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Organic Chemistry

With a Biological Emphasis

Volume II: Chapters 9-17

**Tim Soderberg
University of Minnesota, Morris**



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Notes to the reader:

This textbook is intended for a sophomore-level, two-semester course in Organic Chemistry targeted at Biology, Biochemistry, and Health Science majors. It is assumed that readers have taken a year of General Chemistry and college level Introductory Biology, and are concurrently enrolled in the typical Biology curriculum for sophomore Biology/Health Sciences majors.

Comments and corrections from readers are welcome: please contact the author at **soderbt@morris.umn.edu**

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Chapter 9

Phosphate transfer reactions

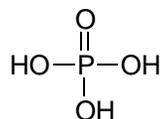


Mono Lake, California

(photo credit <https://www.flickr.com/photos/slolane/>)

Introduction

This chapter is about the chemistry of phosphates, a ubiquitous functional group in biomolecules that is based on phosphoric acid:



phosphoric acid

In late 2010, people around the world found themselves getting a crash course in phosphate chemistry as they watched the evening news. Those who paid close attention to the developing story also got an interesting glimpse into the world of scientific research and debate.

It all started when the American National Aeronautics and Space Administration (NASA) released the following statement to the news media:

“NASA will hold a news conference at 2 p.m. EST on Thursday, Dec. 2, to discuss an astrobiology finding that will impact the search for evidence of extraterrestrial life.”

The wording of the statement attracted widespread media attention, and had some people holding their breath in anticipation that NASA would be introducing a newly discovered alien life form to the world. When December 2nd came, however, those hoping to meet ET were disappointed – the life form being introduced was a bacterium, and it was from our own planet. To biologists and chemists, though, the announcement was nothing less than astounding.

The NASA scientists worked hard to emphasize the significance of their discovery during the news conference. Dr. Felicia Wolfe-Simon, a young postdoctoral researcher who had spearheaded the project, stated that they had “cracked open the door to what's possible for life elsewhere in the universe - and that's profound”. A senior NASA scientist claimed that their results would “fundamentally change how we define life”, and, in attempting to convey the importance of the discovery to a reporter from the newspaper USA Today, referred to an episode from the original Star Trek television series in which the crew of the Starship Enterprise encounters a race of beings whose biochemistry is based on silica rather than carbon.

The new strain of bacteria, dubbed 'GFAJ-1', had been isolated from the arsenic-rich mud surrounding salty, alkaline Mono Lake in central California. What made the strain so unique, according to the NASA team, was that it had evolved the ability to substitute arsenate for phosphate in its DNA. Students of biology and chemistry know that phosphorus is one of the six elements that are absolutely required for life as we know it, and that DNA is a polymer linked by phosphate groups. Arsenic, which is directly below phosphorus on the periodic table, is able to assume a bonding arrangement like that of phosphate, so it might seem reasonable to wonder whether arsenate could replace phosphate in DNA and other biological molecules. Actually finding a living thing with arsenate-linked DNA would indeed be a momentous achievement in biology, as this would represent a whole new chemistry for the most fundamental molecule of life, and

would change our understanding of the chemical requirements for life to exist on earth - and potentially other planets.

In 1987, Professor F.H. Westheimer of Harvard University published what would become a widely read commentary in *Science Magazine* entitled “Why Nature Chose Phosphates”. In it, he discussed the chemical properties that make the phosphate group so ideal for the many roles that it plays in biochemistry, chief among them the role of a linker group for DNA polymers. One of the critical characteristics of phosphate that Westheimer pointed out was that the bonds linking phosphate to organic molecules are stable in water. Clearly, if you are selecting a functional group to link your DNA, you don't want to choose one that will rapidly break apart in water. Among the functional groups that Westheimer compared to phosphate in terms of its suitability as a potential DNA linker was arsenate –but he very quickly dismissed the idea of arsenate-linked DNA because it would be far too unstable in water.

Given this background, it is not hard to imagine that many scientists were puzzled, to say the least, by the NASA results. While the popular media took the announcement at face value and excitedly reported the results as a monumental discovery – NASA is, after all, a highly respected scientific body and the study was being published in *Science Magazine*, one of the most prestigious scientific journals in the world – many scientists quickly voiced their skepticism, mainly in the relatively new and unconstrained venue of the blogosphere. Microbiologist Rosie Redfield of the University of British Columbia, writing in her blog devoted to 'open science', wrote a detailed and highly critical analysis of the study. She pointed out, among other things, that the experimenters had failed to perform the critical purification and mass spectrometry analyses needed to demonstrate that arsenate was indeed being incorporated into the DNA backbone, and that the broth in which the bacteria were being grown actually contained enough phosphate for them to live and replicate using normal phosphate-linked DNA. Science journalist Carl Zimmer, in a column in the online magazine *Slate*, contacted twelve experts to get their opinions, and they were overwhelmingly negative. One of the experts said bluntly, “This paper should not have been published”. Basically, the NASA researchers were making an astounding claim that, if true, would refute decades of established knowledge about the chemistry of DNA – but the evidence they presented was far from convincing. Carl Sagan's widely quoted dictum - “extraordinary claims require extraordinary evidence” - seemed to apply remarkably well to the situation.

What followed was a very public, very lively, and not always completely collegial debate among scientists about the proper way to discuss science: the NASA researchers appeared to dismiss the criticism amassed against their study because it came from blogs, websites, and Twitter feeds. The proper venue for such discussion, they claimed, was in the peer-reviewed literature. Critics countered that their refusal to respond to anything outside of the traditional peer-review system was disingenuous, because they had made full use of the publicity-generating power of the internet and mainstream media in the first place when they announced their results with such fanfare.

The traditional venue for debate, while quite a bit slower than the blogosphere, did eventually come through. When the full paper was published in *Science* a few months later, it was accompanied by eight 'technical comments' from other researchers pointing out deficiencies in the study, an 'editors note', and a broader news article about the controversy. In July of 2012, a paper was published in *Science* under the title "GFAJ-1 Is an Arsenate-Resistant, Phosphate-Dependent Organism". The paper reported definitive evidence that DNA from GFAJ-1, under the conditions described in the NASA paper, did *not* have arsenate incorporated into its structure. Just like professor Westheimer discussed in the 1980s, it appears that nature really did choose phosphate – and only phosphate – after all . . . at least on this planet.

Background reading and viewing:

Youtube video of the NASA press conference:

<http://www.youtube.com/watch?v=WVuhBt03z8g>.

Wolfe-Simon, F. et al. *Science Express*, Dec 2, 2010. The first preview article on the proposed 'arsenic bacteria'.

Wolfe-Simon, F. et al., *Science* **2011**, 332, 1163. The full research paper in *Science Magazine*.

Westheimer, F.H. *Science* **1987**, 235, 1173. The article by Westheimer titled 'Why Nature Chose Phosphates'.

Zimmer, Carl, *Slate*, Dec 7, 2010: Blog post by Carl Zimmer titled 'This Paper Should Not Have Been Published'.

http://www.slate.com/articles/health_and_science/science/2010/12/this_paper_should_not_have_been_published.html

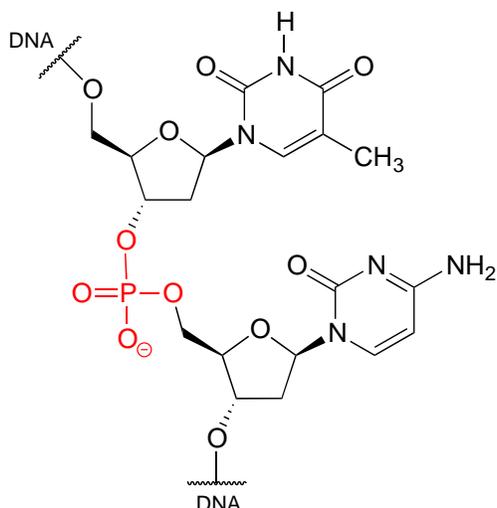
Redfield, R. Blog post Dec 4, 2010:

<http://rrresearch.fieldofscience.com/2010/12/arsenic-associated-bacteria-nasas.html>

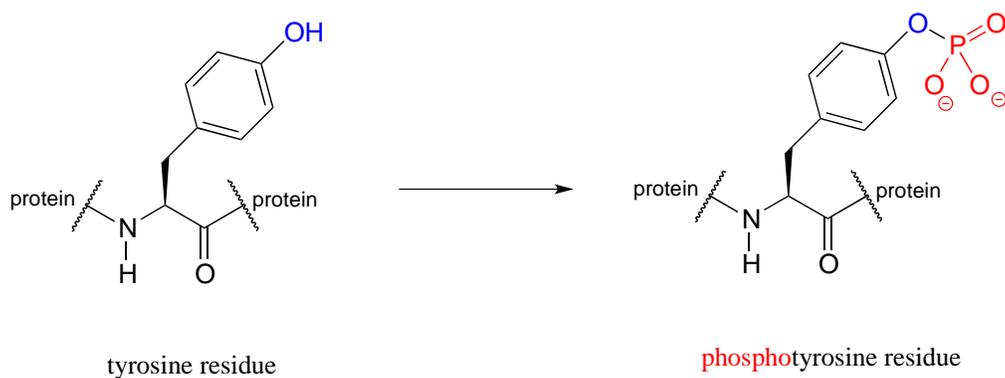
Science **2012**, 337, 467. The paper in *Science Magazine* refuting the validity of the arsenic bacteria claim.

Section 9.1: Overview of phosphate groups

Phosphate is everywhere in biochemistry. As we were reminded in the introduction to this chapter, our DNA is linked by phosphate:



The function of many proteins is regulated - switched on and off - by enzymes which attach or remove a phosphate group from the side chains of serine, threonine, or tyrosine residues.



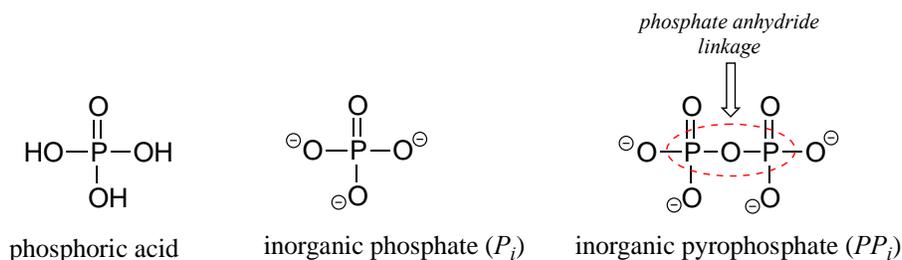
Countless diseases are caused by defects in phosphate transferring enzymes. As just one example, achondroplasia, a common cause of dwarfism, is caused by a defect in an enzyme whose function is to transfer a phosphate to a tyrosine residue in a growth-related signaling protein.

Finally, phosphates are excellent leaving groups in biological organic reactions, as we will see many times throughout the remainder of this book.

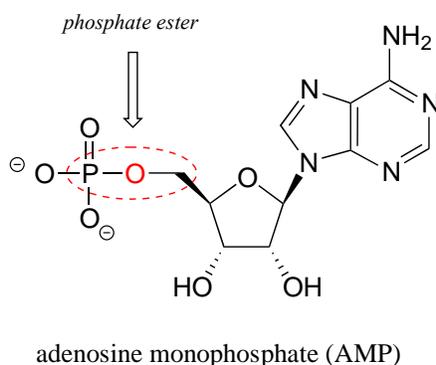
Clearly, an understanding of phosphate chemistry is central to the study of biological organic chemistry. We'll begin with an overview of terms used when talking about phosphates.

9.1A: Terms and abbreviations

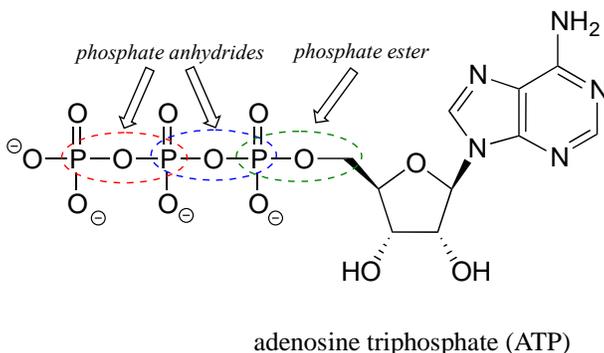
The fully deprotonated conjugate base of phosphoric acid is called a phosphate ion, or **inorganic phosphate** (often abbreviated ' P_i '). When two phosphate groups are linked to each other, the linkage itself is referred to as a '**phosphate anhydride**', and the compound is called '**inorganic pyrophosphate**' (often abbreviated PP_i).



The chemical linkage between phosphate and a carbon atom is a **phosphate ester**. Adenosine monophosphate (AMP) has a single phosphate ester linkage.

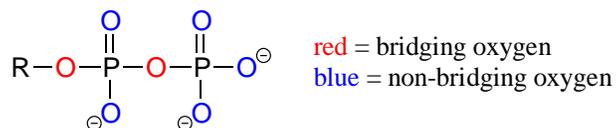


Adenosine triphosphate has one phosphate ester linkage and two phosphate anhydride linkages.

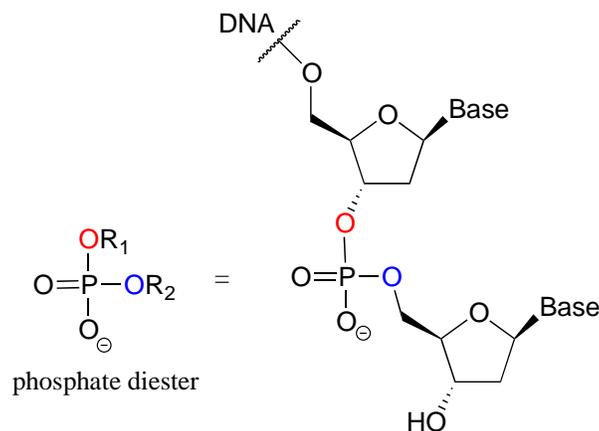


Oxygen atoms in phosphate groups are referred to either as '**bridging**' or '**non-bridging**', depending on their position. An organic diphosphate has two bridging oxygens (one in

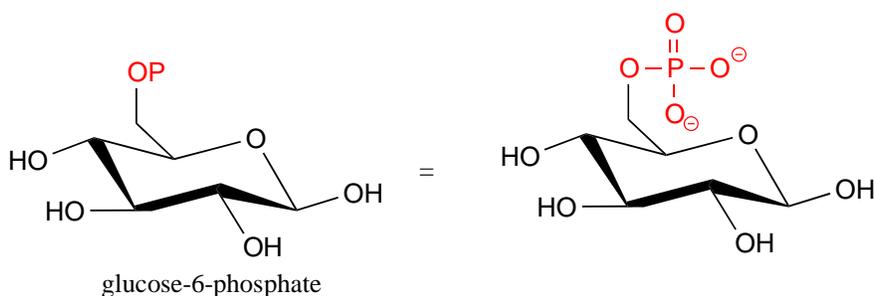
the phosphate ester linkage and one in the phosphate anhydride linkage) and five non-bridging oxygens:



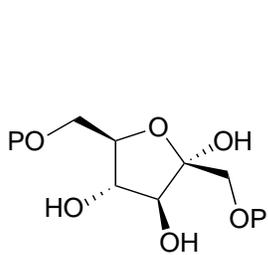
A single phosphate is linked to *two* organic groups is called **phosphate diester**. The backbone of DNA is linked by phosphate diesters.



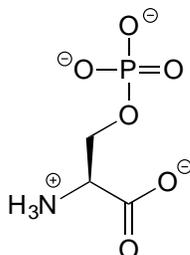
Organic phosphates are often abbreviated using 'OP' and 'OPP' for mono- and diphosphates, respectively. For example, glucose-6-phosphate and isopentenyl diphosphate are often depicted as shown below. Notice that the 'P' abbreviation includes the associated oxygen atoms and negative charges.



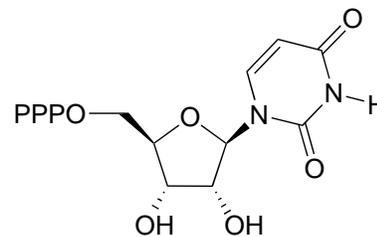
Exercise 9.1: Consider the biological compounds below, some of which are shown with abbreviated structures:



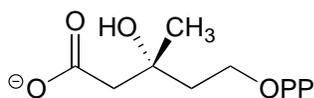
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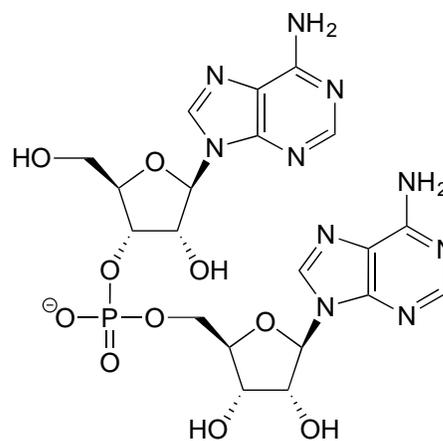
II



III



IV

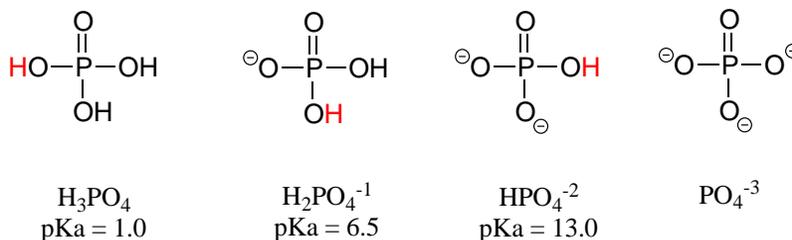


V

- Which contain one or more phosphate anhydride linkages? Specify the number of phosphate anhydride linkages in your answers.
- Which contain one or more phosphate monoesters? Again, specify the number for each answer.
- Which contain a phosphate diester?
- Which could be described as an organic diphosphate?
- For each compound, specify the number of bridging and non-bridging oxygens in the phosphate group.

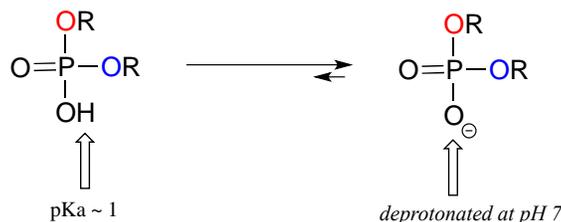
9.1B: Acid constants and protonation states

Phosphoric acid is triprotic, meaning that it has three acidic protons available to donate, with pK_a values of 1.0, 6.5, and 13.0, respectively.



These acid constant values, along with the Henderson-Hasselbalch equation (section 7.2C) tell us that, at the physiological pH of approximately 7, somewhat more than half of the phosphate species will be in the HPO_4^{-2} state, and slightly less than half will be in the $H_2PO_4^{-1}$ state, meaning that the average net charge is between -1.5 and -2.0.

Phosphate diesters have a pK_a of about 1, meaning that they carry a full negative charge at physiological pH.



Organic monophosphates, diphosphates, and triphosphates all have net negative charges and are partially protonated at physiological pH, but by convention are usually drawn in the fully deprotonated state.

Exercise 9.2: Explain why the second pK_a of phosphoric acid is so much higher than the first pK_a .

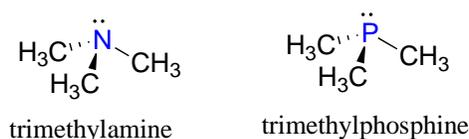
Exercise 9.3: What is the approximate net charge of inorganic phosphate in a solution buffered to pH 1?

Recall from section 8.4 that good leaving groups in organic reactions are, as a rule, weak bases. In laboratory organic reactions, leaving groups are often halides or toluenesulfonates (section 8.4), both of which are weak bases. **In biological organic reactions, phosphates are very common leaving groups.** These could be inorganic

phosphate, inorganic pyrophosphate, or organic monophosphates, all of which are weakly basic, especially when coordinated to metal cations such as Mg^{+2} in the active site of an enzyme. We will see many examples of phosphate leave groups in this and subsequent chapters.

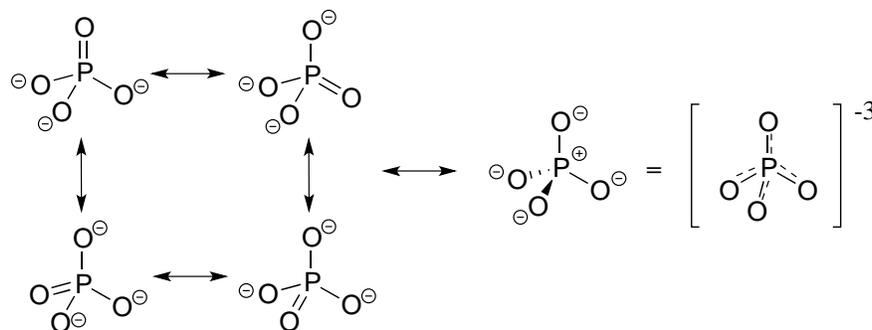
9.1C: Bonding in phosphates

Looking at the location of phosphorus on the periodic table, you might expect it to bond and react in a fashion similar to nitrogen, which is located just above it in the same column. Indeed, **phosphines** - phosphorus analogs of amines - are commonly used in the organic laboratory.



However it is in the form of *phosphate*, rather than phosphine, that phosphorus plays its main role in biology.

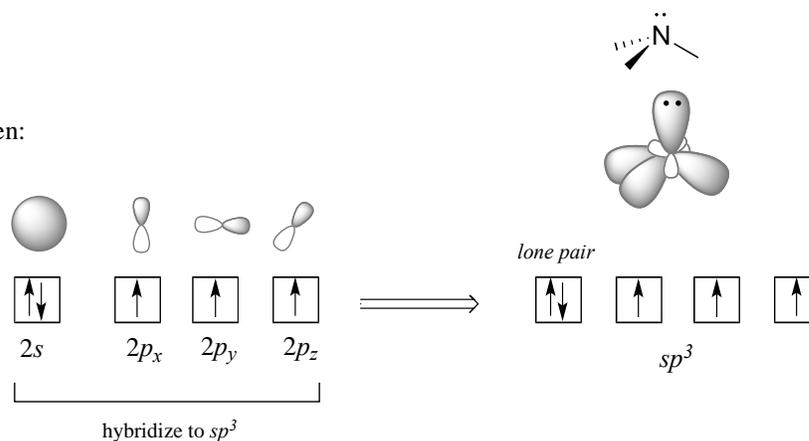
The four oxygen substituents in phosphate groups are arranged about the central phosphorus atom with *tetrahedral geometry*, however there are a total of *five* bonds to phosphorus - four σ bonds and one delocalized π bond.



Phosphorus can break the 'octet rule' because it is on the third row of the periodic table, and thus has *d* orbitals available for bonding. The minus 3 charge on a fully deprotonated phosphate ion is spread evenly over the four oxygen atoms, and each phosphorus-oxygen bond can be considered to have 25% double bond character: in other words, the bond order is 1.25.

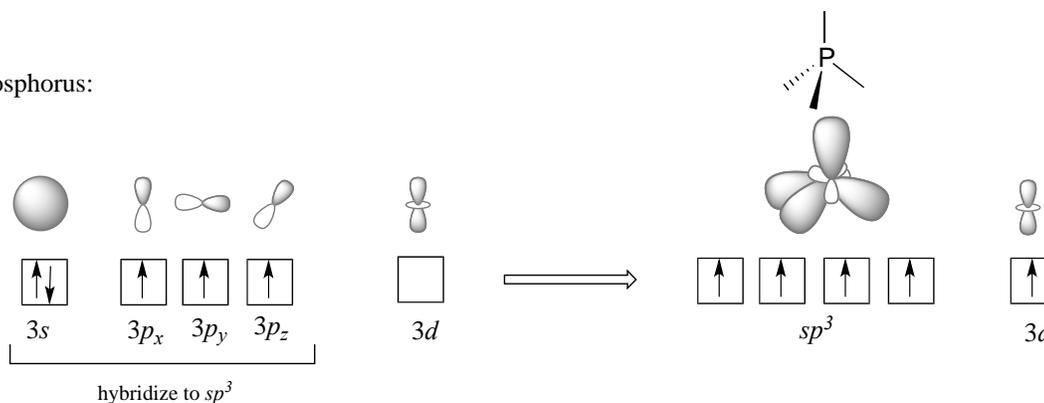
Recall from section 2.1 the hybrid bonding picture for the tetrahedral nitrogen in an amine group: a single $2s$ and three $2p$ orbitals combine to form four sp^3 hybrid orbitals, three of which form σ bonds and one of which holds a lone pair of electrons.

Nitrogen:



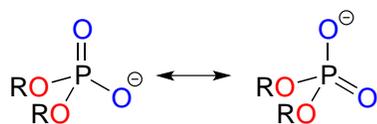
In the hybrid orbital picture for phosphate ion, a single $3s$ and three $3p$ orbitals also combine to form four sp^3 hybrid orbitals with tetrahedral geometry. In contrast to an amine, however, four of the five valence electrons on phosphorus occupy sp^3 orbitals, and the fifth occupies an unhybridized $3d$ orbital.

Phosphorus:

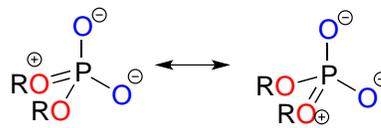
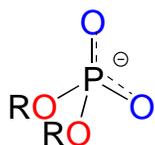


This orbital arrangement allows for four σ bonds with tetrahedral geometry in addition to a fifth, delocalized π bond formed by π overlap between the half-filled $3d$ orbital on phosphorus and $2p$ orbitals on the oxygen atoms.

In phosphate esters, diesters, and anhydrides the π bonding is delocalized primarily over the *non-bridging bonds*, while the bridging bonds have mainly single-bond character. In a phosphate diester, for example, the two non-bridging oxygens share a -1 charge, as illustrated by the two major resonance contributors below. The bonding order for the bridging P-O bonds in a phosphate diester group is about 1, and for the non-bridging P-O bonds about 1.5. In the resonance contributors in which the bridging oxygens are shown as double bonds (to the right in the figure below), there is an additional separation of charge - thus these contributors are minor and make a relatively unimportant contribution to the overall bonding picture.



major resonance contributors

minor resonance contributors
(separation of charge)

non-bridging bonds: significant double bond character

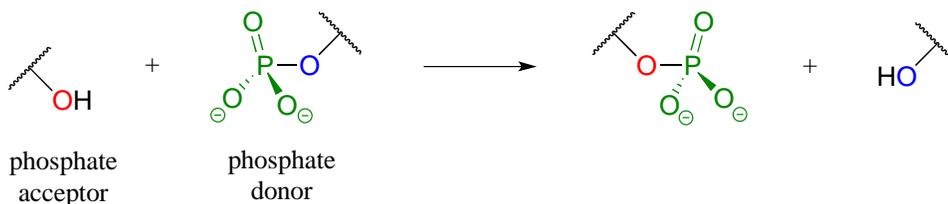
bridging bonds: little double-bond character

Exercise 9.4: Draw all of the resonance structures showing the delocalization of charge on a (fully deprotonated) organic monophosphate. If a 'bond order' of 1.0 is a single bond, and a bond order of 2.0 is a double bond, what is the approximate bond order of bridging and non-bridging P-O bonds?

Throughout this book, phosphate groups will often be drawn without attempting to show tetrahedral geometry, and π bonds and negative charges will usually be shown localized to a single oxygen. This is done for the sake of simplification - however it is important always to remember that the phosphate group is really tetrahedral, the negative charges are delocalized over the non-bridging oxygens, and that there is some degree of protonation at physiological pH (with the exception of the phosphate diester group).

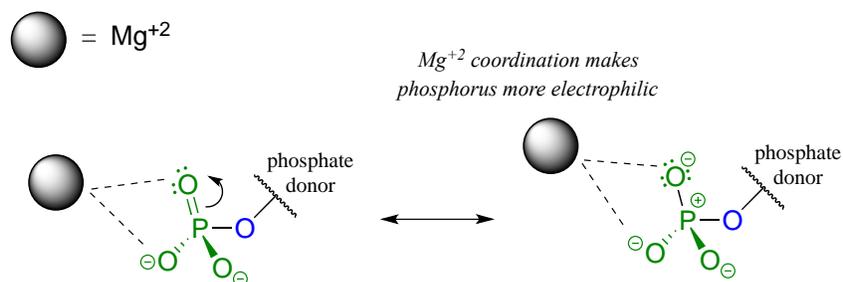
Section 9.2: Phosphate transfer reactions - an overview

In a phosphate transfer reaction, a phosphate group is transferred from a **phosphate group donor** molecule to a **phosphate group acceptor** molecule:



A very important aspect of biological phosphate transfer reactions is that the electrophilicity of the phosphorus atom is usually enhanced by the Lewis acid (electron-accepting) effect of one or more magnesium ions. Phosphate transfer enzymes generally

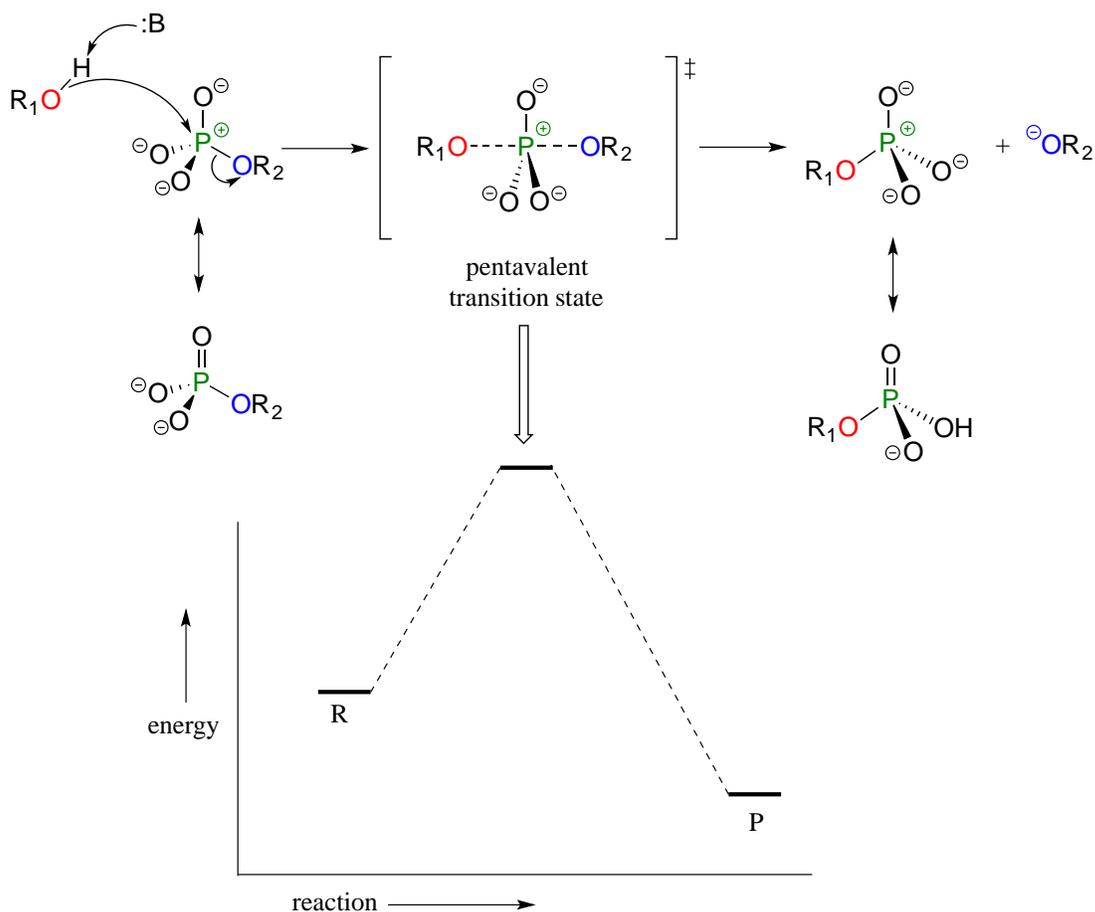
contain a Mg^{2+} ion bound in the active site in a position where it can interact with non-bridging phosphate oxygens on the substrate. The magnesium ion pulls electron density away from the phosphorus atom, making it more electrophilic.



Without this metal ion interaction, a phosphate is actually a poor electrophile, as the negatively-charged oxygens shield the phosphorus center from attack by a nucleophile.

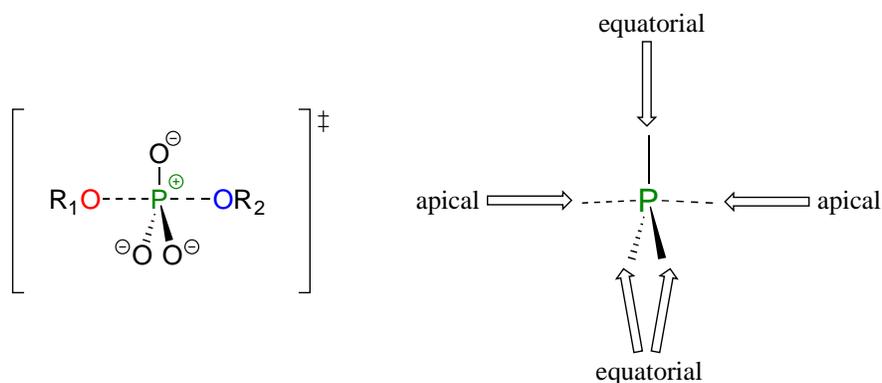
Note: For the sake of simplicity and clarity, we will not draw the magnesium ion or other active site groups interacting with phosphate oxygens in most figures in this chapter - but it is important to keep in mind that these interactions play an integral role in phosphate transfer reactions.

Mechanistically speaking, a phosphate transfer reaction at a phosphorus center can be thought of as much like a $\text{S}_{\text{N}}2$ reaction at a carbon center. Just like in an $\text{S}_{\text{N}}2$ reaction, the nucleophile in a phosphoryl transfer approaches the electrophilic center from the backside, opposite the leaving group:

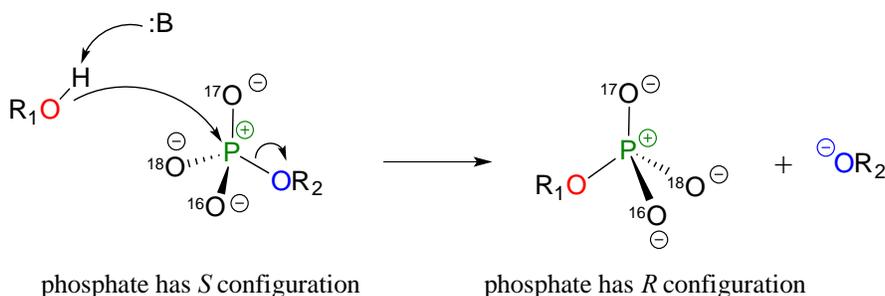
Concerted model:

As the nucleophile gets closer and the leaving group begins its departure, the bonding geometry at the phosphorus atom changes from tetrahedral to trigonal bipyramidal at the pentavalent (5-bond) transition state. As the phosphorus-nucleophile bond gets shorter and the phosphorus-leaving group bond grows longer, the bonding picture around the phosphorus atom returns to its original tetrahedral state, but the stereochemical configuration has been 'flipped', or inverted.

In the trigonal bipyramidal transition state, the five substituents are not equivalent: the three non-bridging oxygens are said to be **equatorial** (forming the base of a trigonal bipyramid), while the nucleophile and the leaving group are said to be **apical** (occupying the tips of the two pyramids).

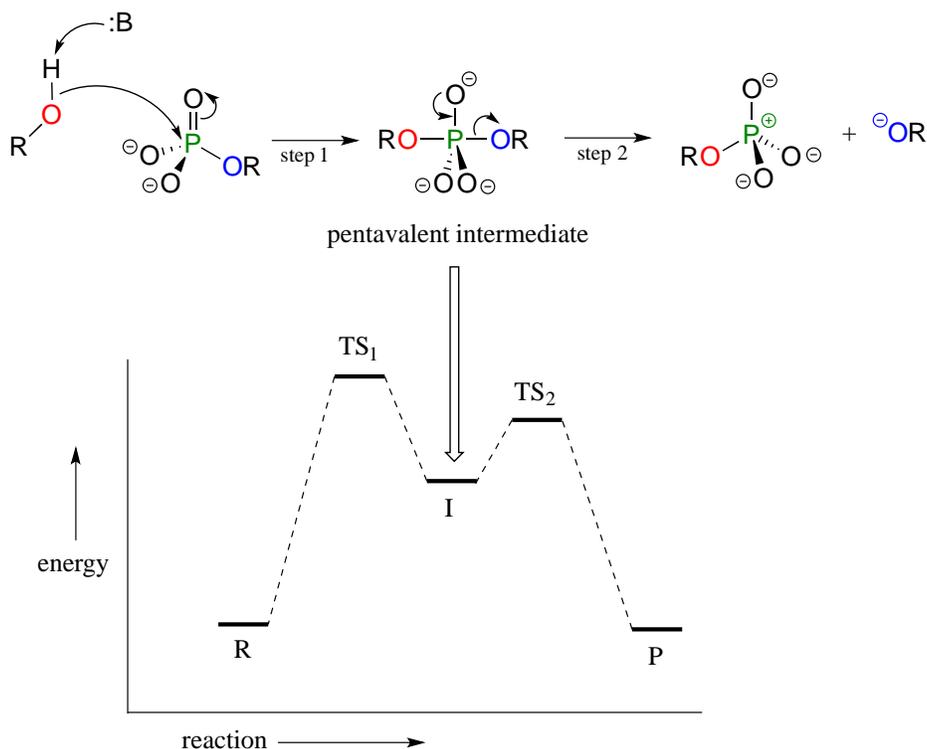


Although stereochemical inversion in phosphoryl transfer reactions is predicted by theory, the fact that phosphoryl groups are achiral made it impossible to observe the phenomenon directly until 1978, when a group of researchers was able to synthesize organic phosphate esters in which stable oxygen isotopes ^{17}O and ^{18}O were specifically incorporated. This created a chiral phosphate center.



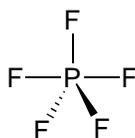
Subsequent experiments with phosphoryl transfer-catalyzing enzymes confirmed that these reactions proceed with stereochemical inversion. (*Nature* **1978** 275, 564; *Ann Rev Biochem* **1980** 49, 877).

The concerted ($\text{S}_{\text{N}}2$ -like) is not the only mechanism that has been proposed for these reactions - in fact, two other possible mechanisms have been suggested. In an alternative two-step mechanistic model, the nucleophile could attack first, forming a pentavalent, trigonal bipyramidal *intermediate* (as opposed to a pentavalent *transition state*). The reaction is completed when the leaving group is expelled. The intermediate species would occupy an energy *valley* between the two transition states.

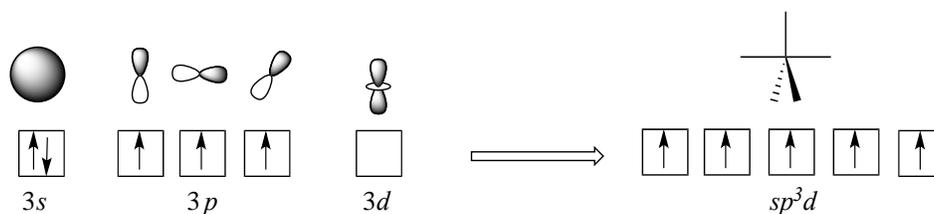
Addition-elimination model:

This is often referred to as an '**addition-elimination**' mechanism - the nucleophile *adds* to the phosphate first, forming a pentavalent intermediate, and then the leaving group is *eliminated*.

An addition-elimination mechanism with a pentavalent intermediate is *not* possible for an S_N2 reaction at a carbon center, because carbon, as a second-row element, does not have any *d* orbitals and cannot form five bonds. Phosphorus, on the other hand, is a third-row element and is quite capable of forming more than four bonds. Phosphorus pentachloride, after all, is a stable compound that has five bonds to chlorine arranged in trigonal bipyramidal geometry around the central phosphorus.

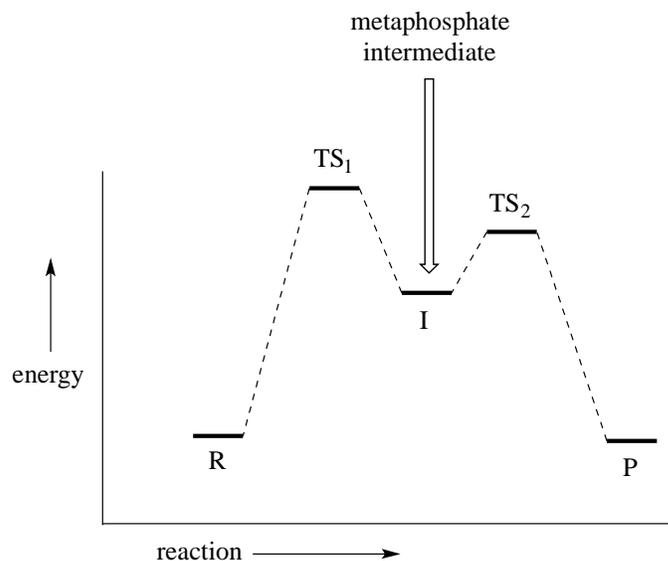
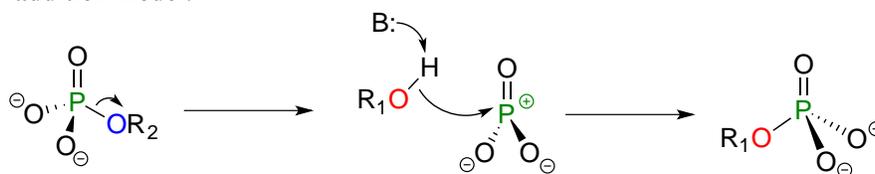


The phosphorus atom in PCl₅ (and in the hypothetical pentavalent intermediate pictured above) is considered to be **sp³d hybridized**:



There is a third possibility: the reaction could proceed in an S_N1 -like manner: in other words, **elimination-addition**. In this model, the phosphorus-leaving group bond breaks first, resulting in a **metaphosphate** intermediate. This intermediate, which corresponds to the carbocation intermediate in an S_N1 reaction and like a carbocation has trigonal planar geometry, is then attacked by the nucleophile to form the reaction product.

Elimination-addition model:



So which mechanistic model - concerted (S_N2 -like), addition-elimination, or elimination-addition - best describes enzymatic phosphate transfer reactions? Chemists love to investigate and debate questions like this! Just like with the S_N1/S_N2 argument discussed in chapter 8, it really boils down to one question: which happens first, bond-forming or bond-breaking - or do these two events occur at the same time? From the evidence accumulated to date, it appears that many enzymatic phosphate transfer reactions can best be described by the concerted model, although there is still argument about this, and still many unanswered questions about other details of how these reactions are catalyzed in active sites. Considering the importance of phosphate transfer reactions in metabolic

pathways, this area is clearly a very promising one for further investigation. If you are interesting in learning more about this research, a great place to start is a review article written by Professor Daniel Herschlag at Stanford University (*Annu. Rev. Biochem.* **2011**, *80*, 669).

For the sake of simplicity and clarity, phosphoryl transfers in this text will be depicted using the concerted model.

Exercise 9.5: Predict the approximate angles between the two bonds indicated in a phosphate transfer transition state. O_a refers to an oxygen at the apical position, and O_e to an oxygen in the equatorial position.

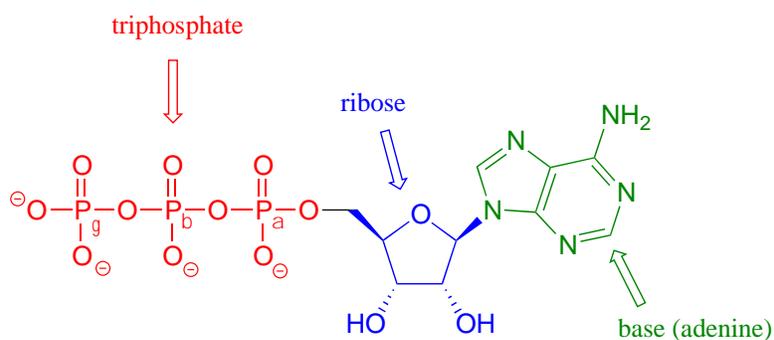
a) O_a -P- O_a

b) O_a -P- O_e

c) O_e -P- O_e

Section 9.3: ATP, the principal phosphate donor

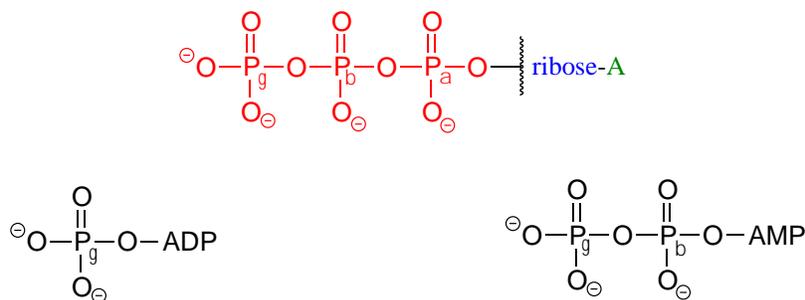
Thus far we have been very general in our discussion of phosphate transfer reactions, referring only to generic 'donor' and 'acceptor' species. It's time to get more specific. The most important donor of phosphate groups in the cell is a molecule called **adenosine triphosphate**, commonly known by its abbreviation **ATP**.



Notice that there are essentially three parts to the ATP molecule: an adenine nucleoside 'base', a five-carbon sugar (ribose), and triphosphate. The three phosphates are designated by Greek letters α , β , and γ , with the α phosphate being the one closest to the ribose. Adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are also important players in the reactions of this chapter.

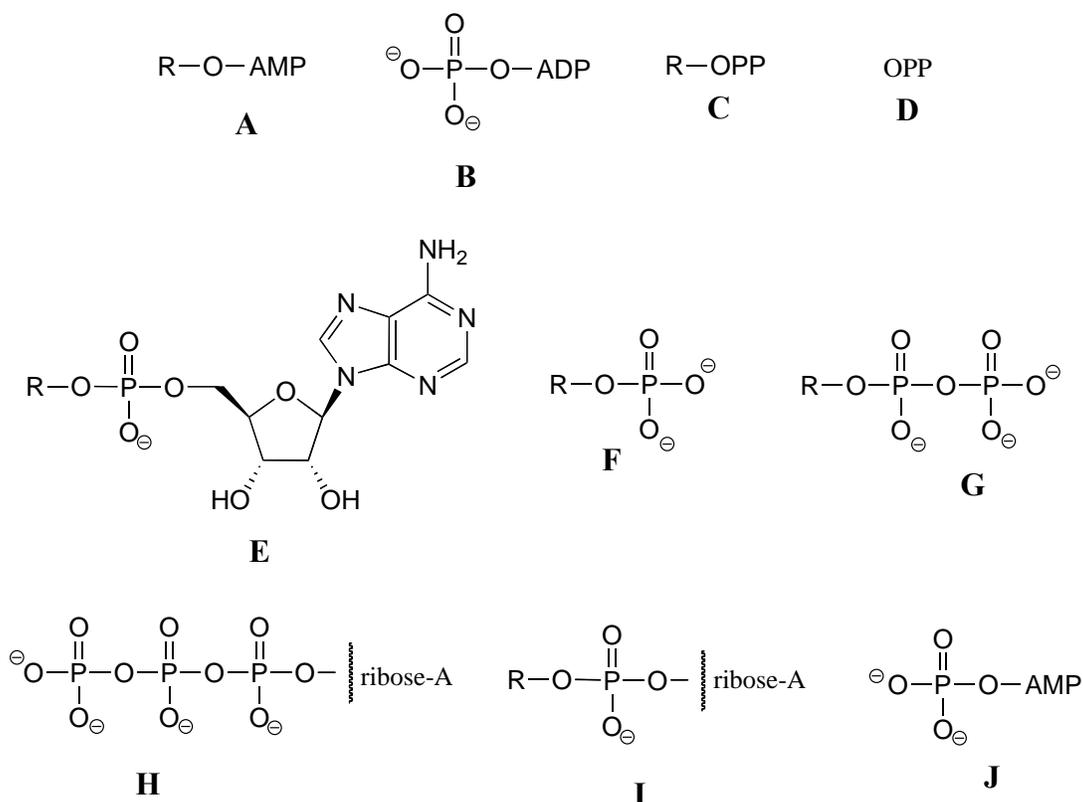
ATP is a big molecule, but the bond-breaking and bond-forming events we will be studying in this chapter all happen in the phosphate part of the molecule. You will see

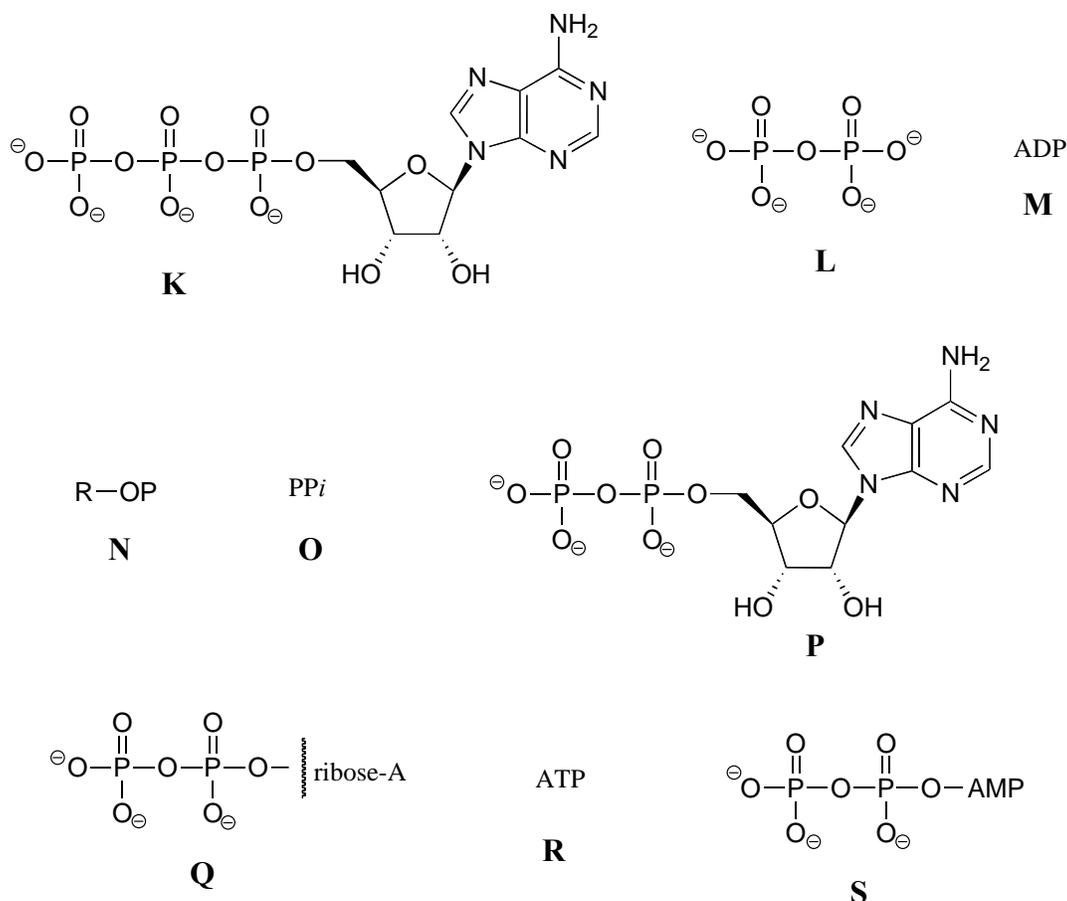
structural drawings of ATP, ADP, and AMP abbreviated in many different ways in this text and throughout the biochemical literature, depending on what is being illustrated. For example, the three structures below are all abbreviated depictions of ATP:



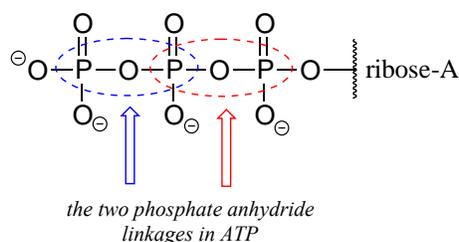
The following exercise will give you some practice in recognizing different abbreviations for ATP and other biological molecules that contain phosphate groups.

Exercise 9.6 : Below are a number of representations, labeled A-S, of molecules that contain phosphate groups. Different abbreviations are used. Arrange A-S into groups of drawings that depict the same species (for example, group together all of the abbreviations which depict ATP).

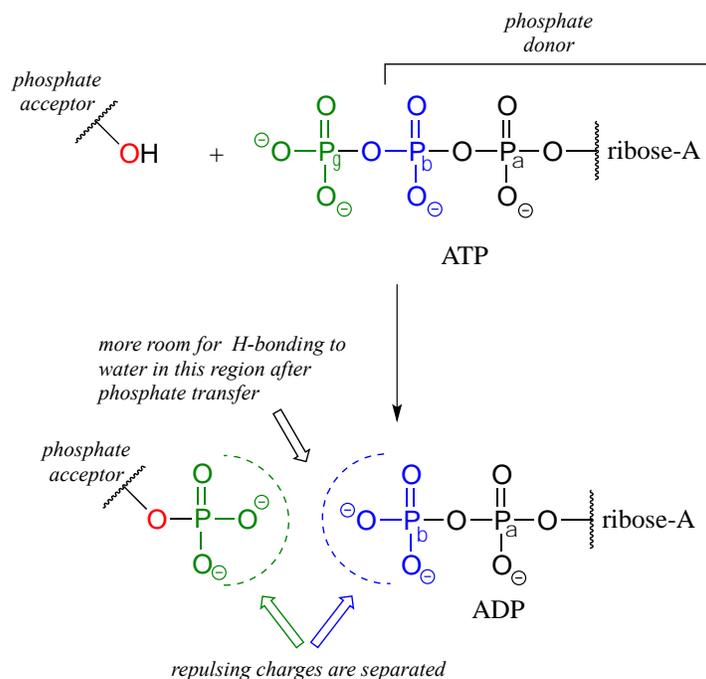




You are probably familiar with the physiological role of ATP from your biology classes - it is commonly called 'the energy currency of the cell'. What this means is that ATP stores energy we get from the oxidation of fuel molecules such as carbohydrates or fats. The energy in ATP is stored in the two high-energy phosphate anhydride linkages.



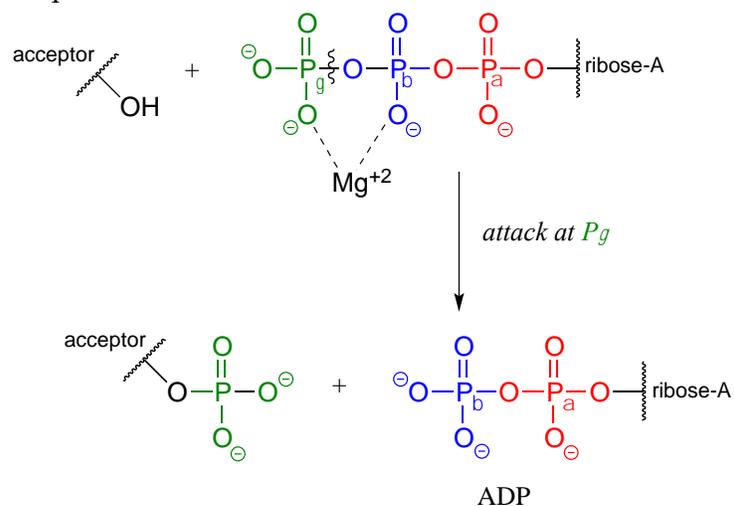
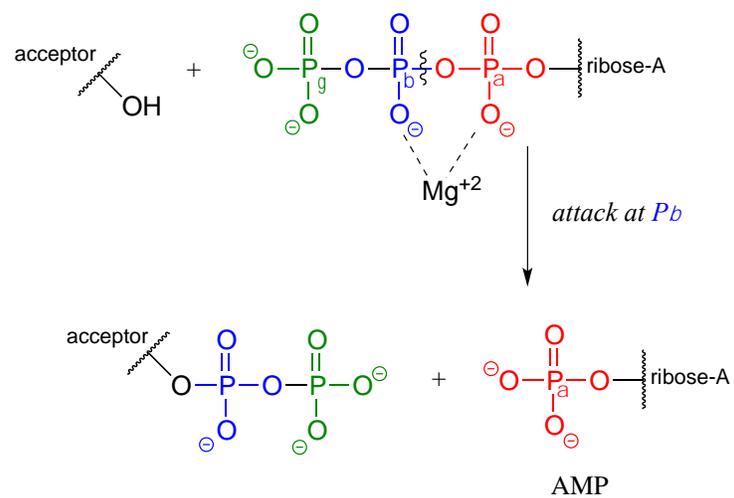
When one or both of these phosphate anhydride links are broken as a phosphate group is transferred to an acceptor, a substantial amount of energy is released. The negative charges on the phosphate groups are separated, eliminating some of electrostatic repulsion that existed in ATP. One way to picture this is as a coil springing open, releasing potential energy.



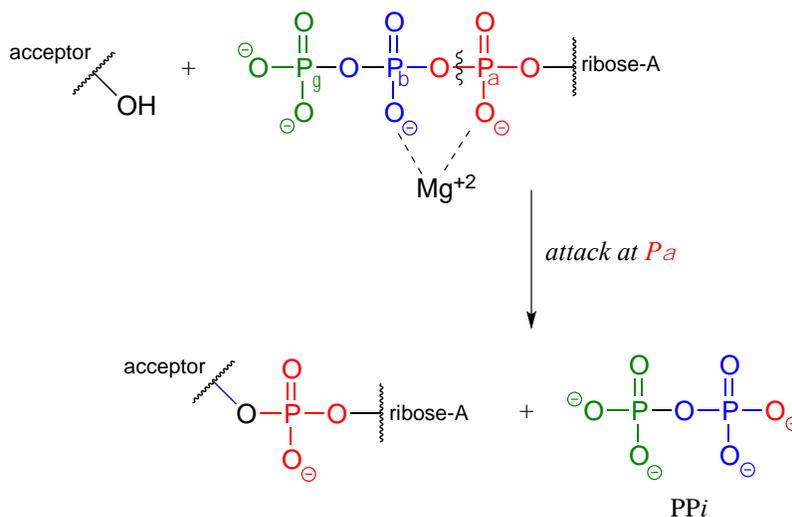
In addition, cleavage of a phosphate anhydride bond means that surrounding water molecules are able to form more stabilizing hydrogen-bonding interactions with the products than was possible with the starting materials, again making the reaction more 'downhill', or exergonic.

It is important to understand that while the phosphate anhydride bonds in ATP are *thermodynamically* unstable (they contain a great deal of chemical energy), they are at the same time *kinetically* stable: ATP-cleaving reactions are exothermic, but also have a high energy barrier, making them very slow *unless catalyzed by an enzyme*. In other words, the release of the energy contained in ATP is highly energetic but also subject to tight control by the interaction of highly evolved enzymes in our metabolic pathways.

ATP is a versatile phosphate group donor: depending on the site of nucleophilic attack (at the α , β , or γ phosphorus), different phosphate transfer outcomes are possible. Below are the three most common patterns seen in the central metabolic pathways. A 'squigly' line in each figure indicates the P-O bond being broken. We will study specific examples of each of these in the coming sections.

Attack at the γ -phosphate:Attack at the β -phosphate:

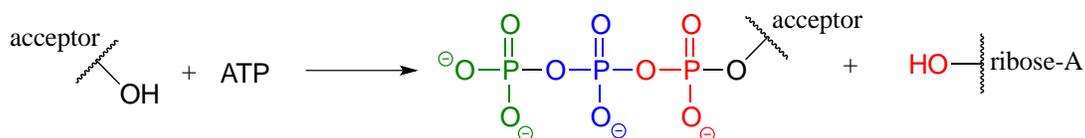
Attack at the α -phosphate:



The common thread running through all of the ATP-dependent reactions we will see in this section is the idea that the phosphate acceptor molecule is undergoing a thermodynamically 'uphill' transformation to become a more reactive species. The energy for this uphill transformation comes from breaking a high-energy phosphate anhydride bond in ATP. That is why ATP is often referred to as 'energy currency': the energy in its anhydride bonds is used to 'pay for' a thermodynamically uphill chemical step.

Exercise 9.7: Propose a fourth hypothetical phosphate transfer reaction between ATP and the generic acceptor molecule in the figure above, in which inorganic phosphate (P_i) is a by-product.

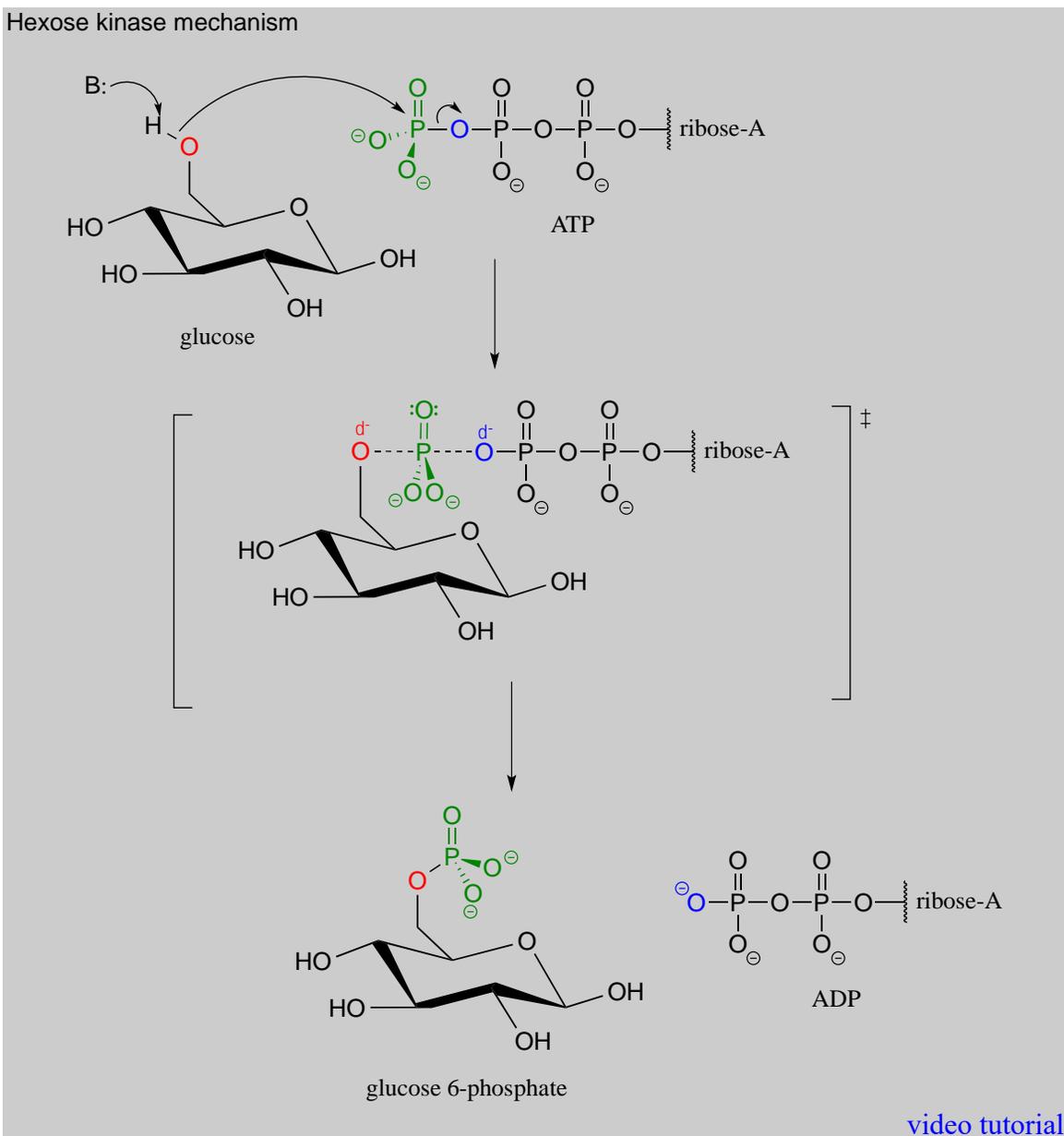
Exercise 9.8: Why is this hypothetical phosphate transfer reaction less energetically favorable compared to all of the possible ATP-cleaving reactions shown in the figure above?



Section 9.4: Phosphorylation of alcohols

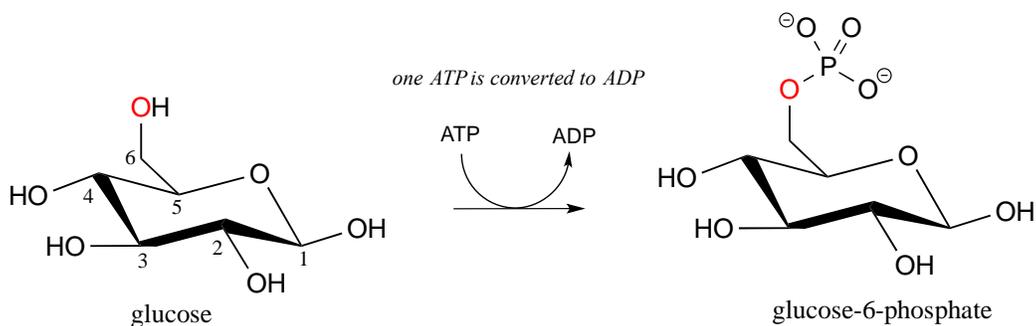
A broad family of enzymes called **kinases** catalyze transfer of a phosphate group from ATP to an alcohol acceptor. Mechanistically, the alcohol oxygen acts as a nucleophile, attacking the electrophilic γ -phosphorus of ATP and expelling ADP.

Glucose is phosphorylated in the first step of the glycolysis pathway by the enzyme hexose kinase (EC 2.7.1.1), forming glucose-6-phosphate.

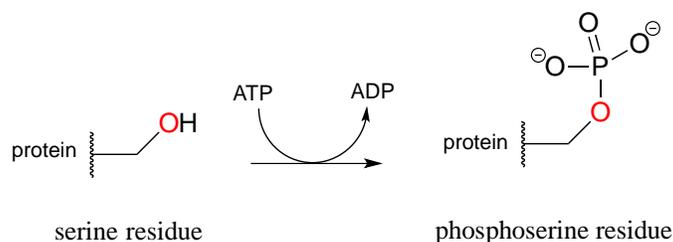


Here is a shorthand way to depict this reaction. Notice the "ATP in, ADP out" notation used below, indicating that ATP is one of the reactants and ADP is one of the products.

From here on, we will frequently use this common convention to indicate reaction participants whose structures are not drawn out in a figure.



The biological activity of many proteins is regulated by **protein kinases**. In a protein kinase reaction, the side chain hydroxyl groups on serine, threonine, or tyrosine residues of certain proteins are phosphorylated by ATP:

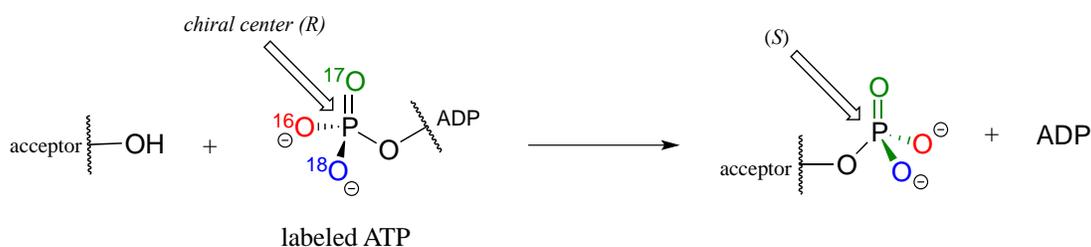


The conversion of a neutral hydroxyl group to a charged phosphate represents a very dramatic change in the local architecture of the protein, potentially altering its folding pattern and ability to bind to small molecules or other proteins. A protein's biological function can be 'switched on' by phosphorylation of a single residue, and switched off again by removal of the phosphate group. The latter reaction we will examine later in this chapter.

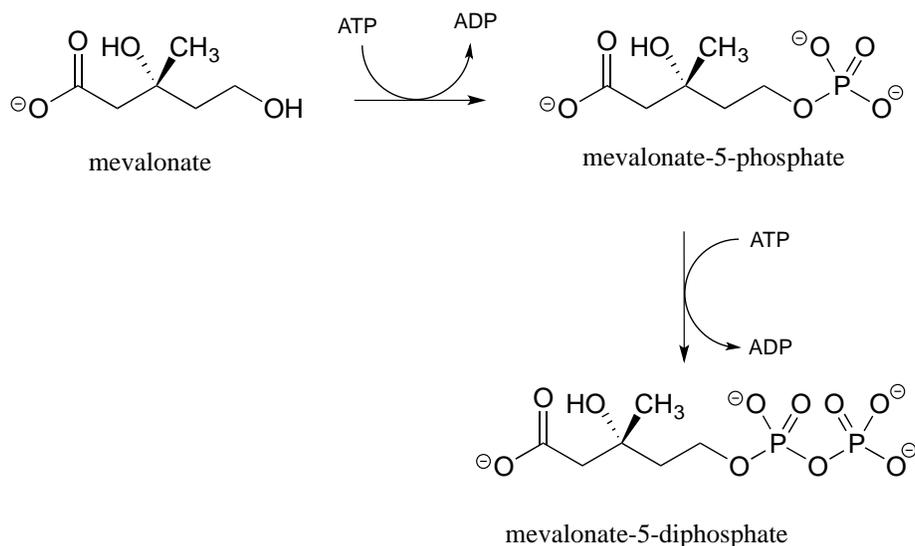
Exercise 9.9:

- Draw a curved-arrow mechanism, using abbreviations as appropriate, for the serine kinase reaction.
- Threonine kinase catalyzes the phosphorylation of the side chain hydroxyl group of threonine residues in proteins. Draw the structure, including the configuration of all stereocenters, of a phosphothreonine residue. Explain how you can predict the stereochemistry of the side chain.

Although stereochemical inversion in phosphate transfer is predicted by theory, the fact that phosphate groups are achiral made it impossible for a long time to verify the phenomenon directly. This was finally accomplished in the late 1970's, when a group of researchers demonstrated phosphate inversion in kinase enzymes using chemically synthesized ATP in which three different isotopes of oxygen were incorporated into the γ -phosphate, thus creating a chiral phosphorus center. (*Ann. Rev. Biochem.* **1980** 49, 877)

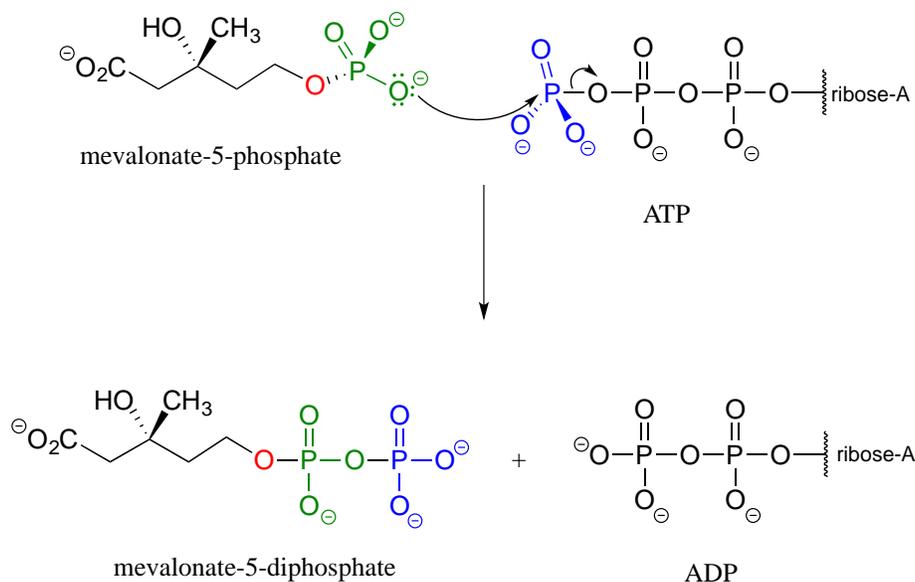


Alcohols can be converted into organic **diphosphates** in two different ways. A two-step process simply involves successive transfers of the γ -phosphate groups of two ATP donors, such as in these sequential steps in isoprenoid biosynthesis. (EC 2.7.1.36; EC 2.7.4.2). A compound called mevalonate is diphosphorylated in this way in the early phase of the biosynthetic pathway for cholesterol, steroid hormones, and other isoprenoid molecules.



The mechanism for the first phosphorylation step is analogous to that for an alcohol kinase reaction, which we have just seen. In the second phosphate transfer step,

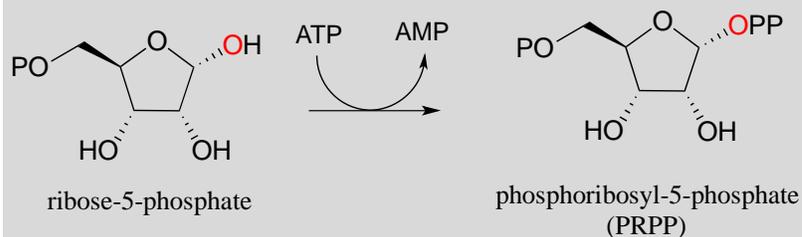
catalyzed by a separate enzyme, one of the phosphate oxygens on the organic monophosphate acts as a nucleophilic phosphate acceptor, attacking the γ -phosphate of a second ATP.



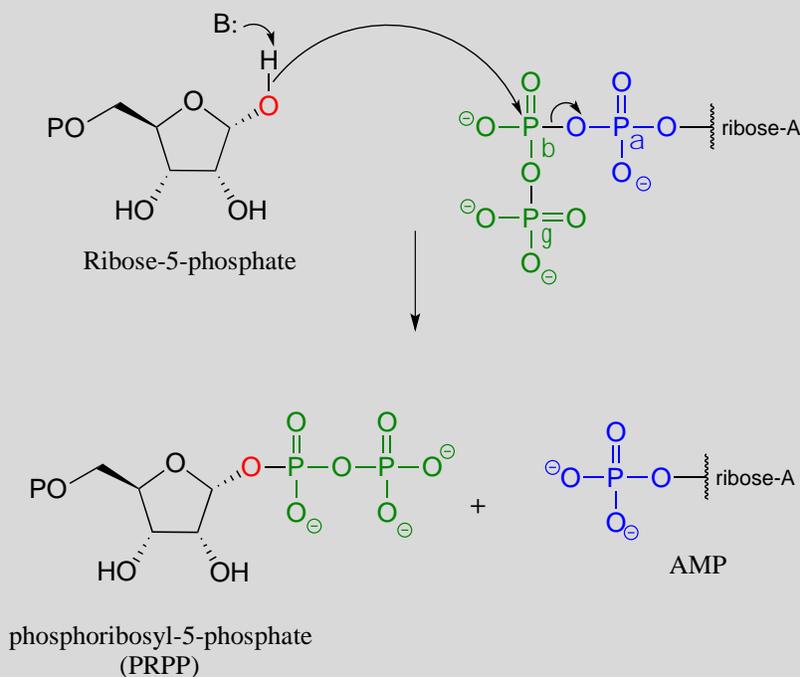
In some metabolic pathways, diphosphorylation occurs by a different mechanism from the one above. Instead of sequentially transferring two phosphates from two ATP donors, the alternate mechanism occurs in a single step: the nucleophilic acceptor molecule attacks the β -phosphate of ATP, rather than the γ -phosphate. After formation of the trigonal bipyramidal intermediate, it is AMP (not ADP) which is expelled, and what started out as the β and γ phosphates of ATP both remain with the acceptor.

In the biosynthesis of DNA and RNA nucleotides, one of the hydroxyl groups on ribose-5-phosphate is diphosphorylated (EC 2.7.6.1) in a one-step mechanism:

A one-step alcohol diphosphorylation reaction (PRPP synthase):



Mechanism:



The metabolic role of both of the diphosphorylation processes we just saw is to convert a hydroxyl group into a good leaving group (recall that hydroxide ions are strong bases and poor leaving groups, while phosphates/diphosphates, especially when stabilized in an enzyme active site, are weak bases and very good leaving groups). In nucleoside biosynthesis pathways, the diphosphate group of PRPP acts as a leaving group in the very next metabolic step, which is an S_N1 reaction: we have already seen this reaction in section 8.7B).

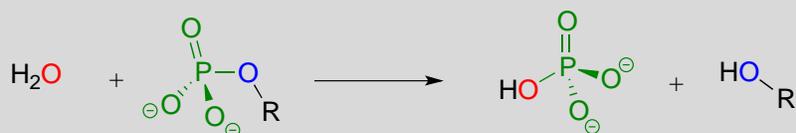
Section 9.5: Phosphorylation of carboxylates

Thus far we have seen hydroxyl oxygens and phosphate oxygens acting as nucleophilic accepting groups in ATP-dependent phosphate transfer reactions. Carboxylate oxygens can also accept phosphate groups from ATP. This typically happens in two different ways. First, the carboxylate can attack the γ -phosphate of ATP to accept phosphate, generates a species known as an 'acyl phosphate'. An example is the first part of the reaction catalyzed by glutamine synthase (EC 6.3.1.2):

Section 9.6: Hydrolysis of organic phosphates

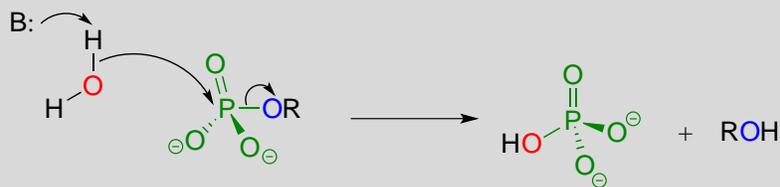
While kinase enzymes catalyze the phosphorylation of organic compounds, enzymes called **phosphatases** catalyze *dephosphorylation* reactions. **The reactions catalyzed by kinases and phosphatases are *not* the reverse of one another:** kinases irreversibly transfer phosphate groups from ATP (or sometimes other nucleoside triphosphates) to various organic acceptor compounds, while *phosphatases transfer phosphate groups from organic compounds to water*: these are *hydrolysis* reactions. Kinase reactions involve an inherently 'uphill' step (phosphorylation of an alcohol, for example) being paid for with an inherently 'downhill' step (cleavage of an anhydride bond in ATP). Phosphatase reactions, on the other hand, are thermodynamically 'downhill', and while they require an enzyme to speed them up, they do not involve 'spending' energy currency the way kinase reactions do.

Phosphatase reaction:

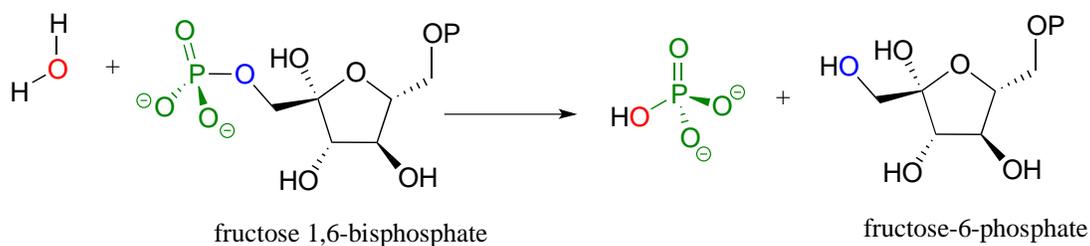


There are two possible general mechanisms for a phosphatase reaction. Some enzymes catalyze *direct* hydrolysis reactions, in which the phosphate group is removed by direct attack of a water molecule at the phosphate center:

Phosphatase mechanism (direct hydrolysis):



One of the two phosphate groups on fructose 1,6-bisphosphate is hydrolyzed in such a way late in the gluconeogenesis pathway. (*Biochemistry* 2000, 39, 8565; EC 3.1.3.11)



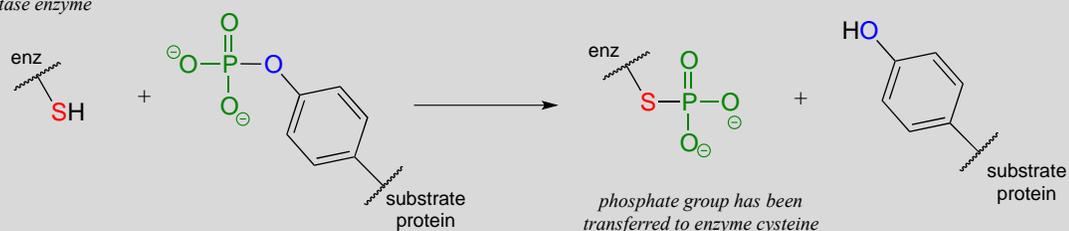
Many phosphatase reactions, however, operate by a slightly more complicated mechanism than what is shown above. In the first phase, a nucleophilic enzyme group (typically a cysteine, aspartate, glutamate, or histidine side chain, designated in the figure below as 'X') attacks the phosphate group. In the second phase, the phosphorylated residue is hydrolyzed. For example, protein tyrosine phosphatase catalyzes the dephosphorylation of phosphotyrosine residues in some proteins - this is the other half of the regulatory 'on-off switch' that we discussed earlier in the context of protein kinases. In the first step, the phosphate group is directly donated to a cysteine side chain in the phosphatase enzyme's active site. In the second step, the phosphocysteine intermediate is cleaved by water to form inorganic phosphate and regenerate the free cysteine in the active site.

Indirect phosphatase reaction:

Step 1:

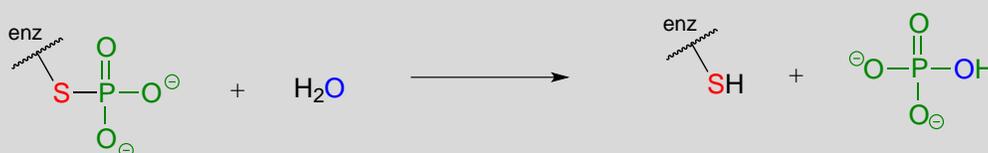
active site cysteine on
phosphatase enzyme

phosphotyrosine on substrate protein



Step 2:

free cysteine is regenerated

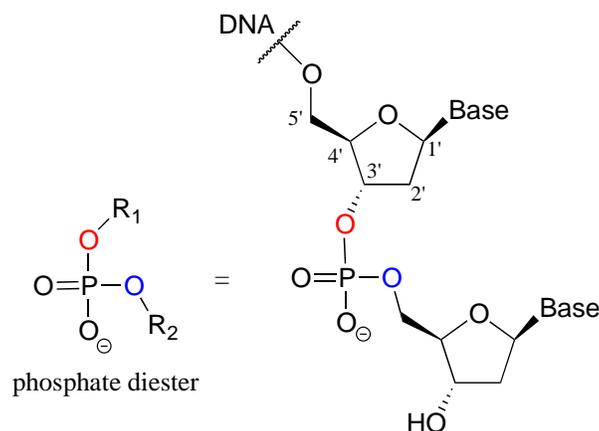


Notice that in the end, the phosphate group has still been transferred to a water molecule, albeit indirectly. How would you know, just by looking at the substrate and product of the protein tyrosine phosphatase reaction, that the phosphate is not transferred *directly* to a water molecule? Simply put, you *wouldn't* know this information without the benefit of knowledge gained from biochemical experimentation.

Exercise 9.11: If you were to look just at the substrates and products of a phosphatase reaction without knowing anything about the mechanism, it is apparent that a *nucleophilic substitution* mechanism could theoretically account for the products formed. Draw out a hypothetical nucleophilic substitution mechanism for the hydrolysis of a phosphoserine residue and show how researchers, by running the reaction in H_2^{18}O , (isotopically labeled water), could potentially distinguish between a nucleophilic substitution and phosphate group transfer mechanism by looking at where the ^{18}O atom ends up in the products.

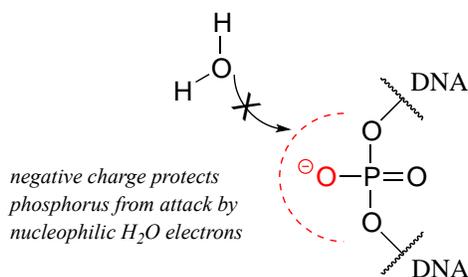
Section 9.7: Phosphate diesters in DNA and RNA

Phosphate diesters play an absolutely critical role in nature - they are the molecular 'tape' that connect the individual nucleotides in DNA and RNA *via* a sugar-phosphate backbone. Take note of the 1' - 5' carbon numbering shown below for the ribose sugar - these numbers will be used frequently in the coming discussion. The 'prime' symbol (') is used to distinguish the ribose carbon numbers from the nucleotide base carbon numbers (which are not shown here).



The introduction to this chapter referenced a widely-read 1987 commentary in *Science Magazine*, in which F.H. Westheimer of Harvard University addressed the question of why phosphates were 'chosen' by nature for critical biochemical job of linking DNA (*Science* **1987**, 235, 1173). He emphasizes how critical it is for the phosphate diester linkage in DNA to be stable in water – in other words, it must be resistant to spontaneous (nonenzymatic) hydrolysis. Even very infrequent occurrence of such an undesired hydrolysis event could be disastrous for an organism, given that an intact DNA strand is a *long-term* storage mechanism for genetic information.

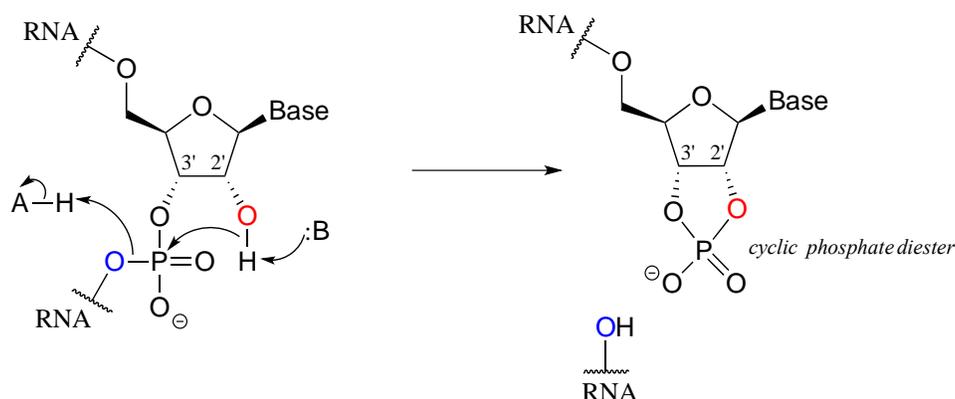
Westheimer pointed out that the inherent stability of DNA is due in large part to the negative charge on the non-bridging oxygen of the phosphate diester linker, which effectively repels nucleophilic water molecules and shields the electrophilic phosphorus atom from attack.



While DNA is quite stable with regard to spontaneous hydrolysis, it of course can be degraded by specific *enzymatic* hydrolysis, where the phosphate electrophile is activated for attack through noncovalent interactions (eg. with Mg²⁺) in the active site. Enzymes that hydrolyze the phosphate diester bonds in DNA are called nucleases, and we will learn more about them in section 9.8.

Unlike DNA, RNA is quite vulnerable to spontaneous hydrolysis in aqueous solution. This does not present a physiological dilemma, because the function of RNA is to encode genetic information on a temporary rather than long-term basis. Why does hydrolysis occur so much more rapidly in RNA than in DNA? The answer has everything to do

with the lowered *entropic* barrier to the reaction (you might want to quickly review the concept of entropy at this point). RNA nucleotides, unlike the *deoxynucleotides* of DNA, have a hydroxyl group at the neighboring 2' carbon. The 2' hydroxyl group is right next to the electrophilic phosphorus atom, poised in a good position to make a nucleophilic attack, breaking the RNA chain and forming a cyclic phosphate diester intermediate (see figure below).



Researchers working with RNA have to be careful to store their samples at very cold temperatures, preferably freeze-dried or precipitated in ethanol, to avoid hydrolysis. The problem of RNA decomposition is compounded by the fact that RNAase enzymes, which catalyze RNA hydrolysis, are present on the surface of human skin and are very stable, long-lived, and difficult to destroy.

In contrast, DNA samples can be safely stored in aqueous buffer in a refrigerator, or in a freezer for longer-term storage.

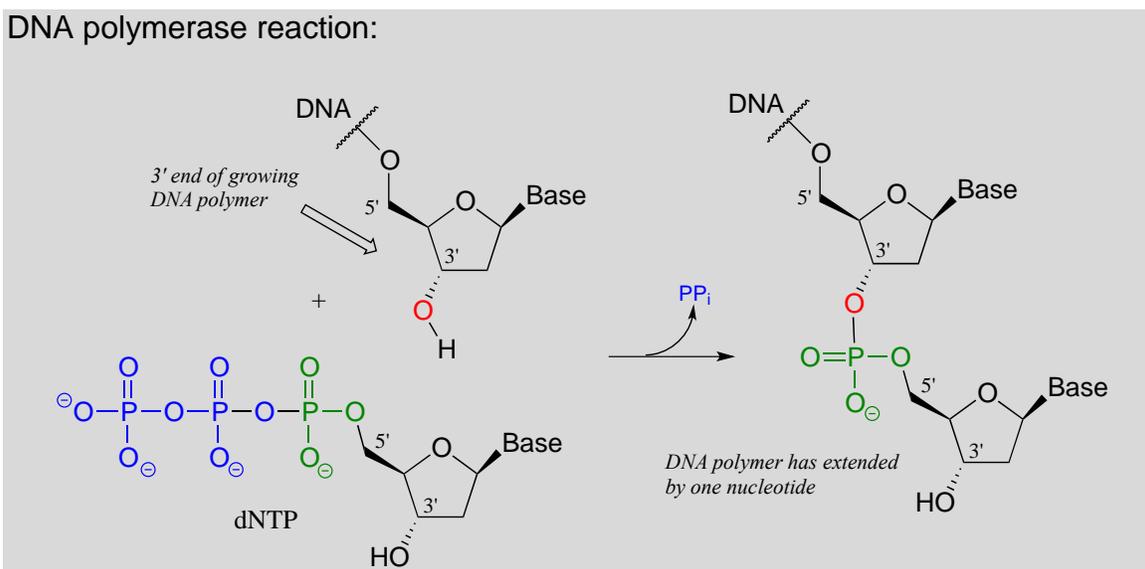
Section 9.8: The organic chemistry of genetic engineering

Many enzymes that catalyze reactions involving the phosphate diester bonds of DNA have been harnessed for use in genetic engineering - techniques in which we copy, snip, and splice DNA in order to create custom versions of genes. The tools of genetic engineering have become indispensable and commonplace in the past decade, and most researchers working on the biological side of chemistry use them extensively. The days of painstakingly purifying an enzyme from bacterial cultures or ground-up cow livers are pretty much gone. Now scientists clone the gene that encodes the enzyme, make any desired changes (by site-directed mutagenesis, for example), and use a host such as *E. coli* or yeast to produce 'recombinant' enzyme from the cloned gene. You will learn the details of many of these procedures in a biochemistry or molecular biology course. What we will focus on now is applying what we have learned about phosphate group transfer reactions so that we can recognize some of the organic chemistry that is happening in a cloning experiment.

The first thing you have to do in a gene cloning procedure is to copy a DNA strand. This is accomplished by an enzyme called **DNA polymerase** (EC 2.7.7.7), which uses a single strand of DNA as a template to synthesize a second, complementary strand (the full picture of this complex process is well beyond the scope of this book, but recall that we talked about the discovery of thermostable DNA polymerase in the introduction to chapter 6).

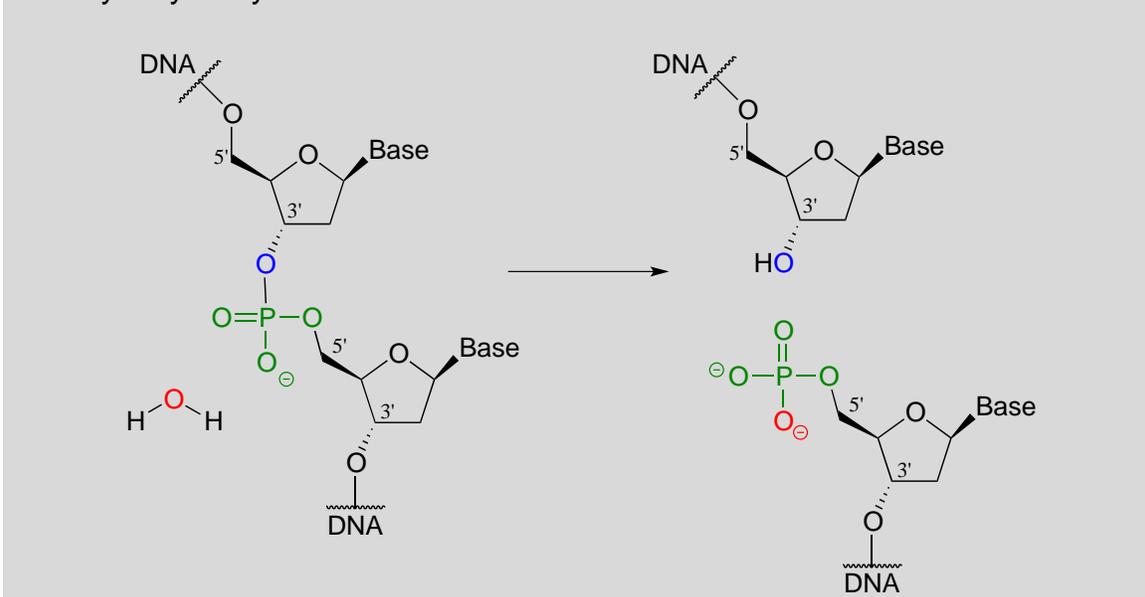
You may have learned in a biology class that DNA is synthesized in the 3' to 5' direction. Notice below that the 3' hydroxyl group on the end of the growing DNA strand attacks the α -phosphate of a 2'-deoxynucleoside triphosphate (dNTP), expelling inorganic pyrophosphate.

DNA polymerase reaction:



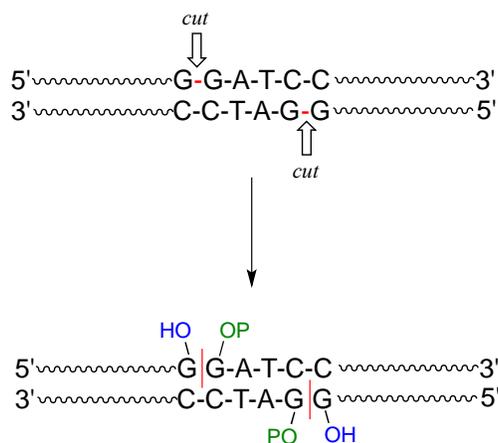
Scientists are able to cut DNA using 'molecular scissor' enzymes called **restriction endonucleases** that cleave double-stranded DNA by hydrolysis at specific base sequences.

DNA hydrolysis by restriction endonucleases:



Notice that the result of this hydrolytic cleavage reaction is one segment of DNA with a hydroxy group at the 3' position, and a second segment with a phosphate group at the 5' position.

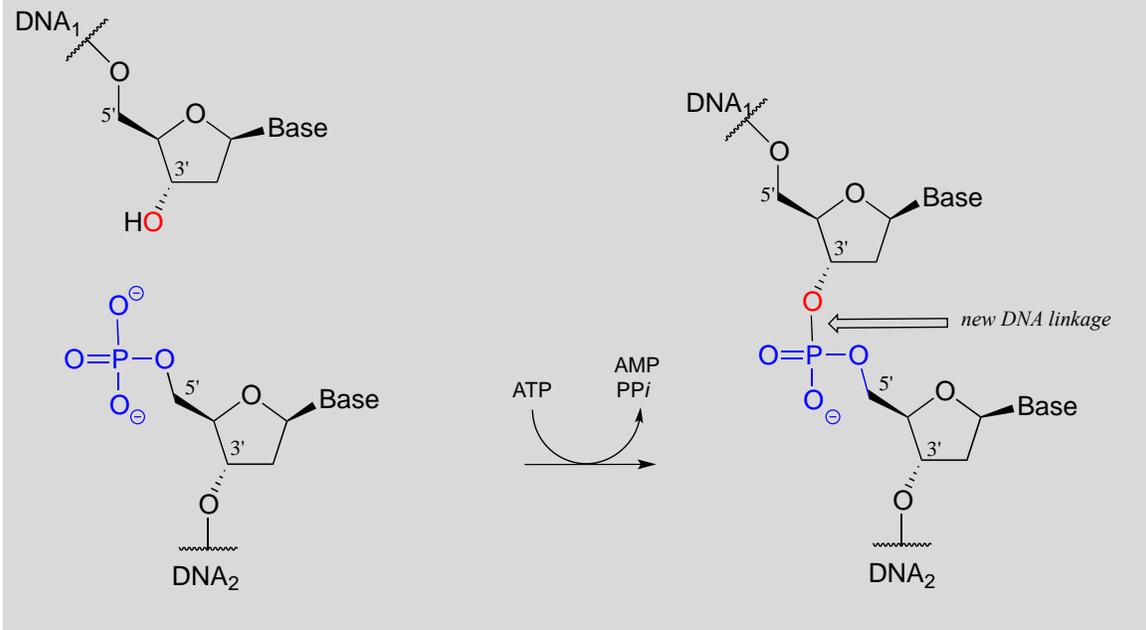
A commonly used restriction endonuclease called 'BamHI' cleaves double-stranded DNA specifically at the following 6-base sequence:



Notice that a 'staggered' cut is made: this is a common (and useful) property of many endonucleases, although some make 'blunt-ended' cuts.

While an endonuclease cleaves a phosphodiester linkage in a DNA strand, **DNA ligase** (EC 6.5.1.1) accomplishes the reverse process: it catalyzes the formation of a new 3'-5' link between two strands:

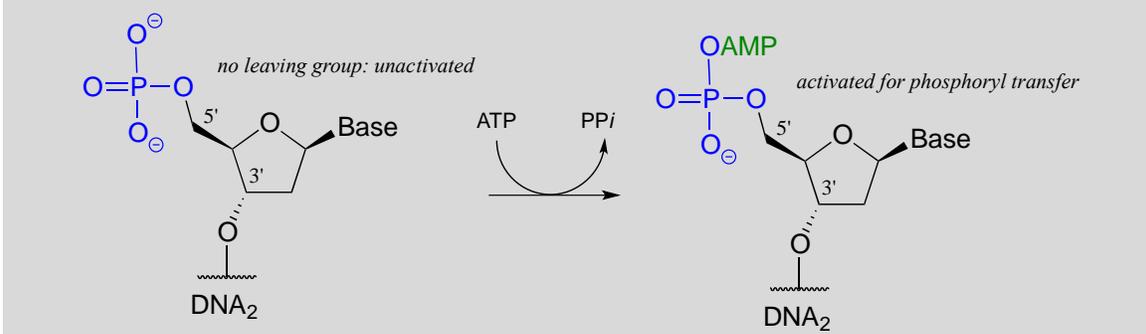
DNA ligase reaction:



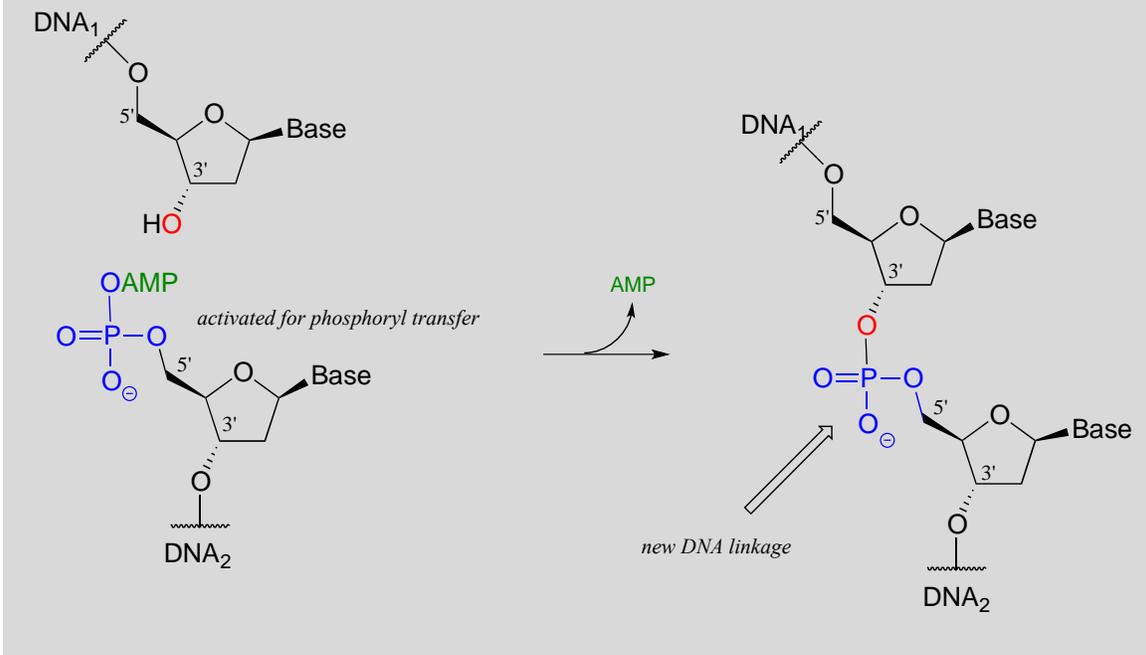
Note that there is initially no leaving group on the 5' phosphate of DNA₂, which makes a direct phosphate transfer reaction impossible. The strategy employed by the DNA ligase enzyme is to first *activate* the 5' phosphate of DNA₂ using ATP (phase 1 below), then the ligation reaction can proceed (phase 2)

DNA ligation

Phase 1: activation

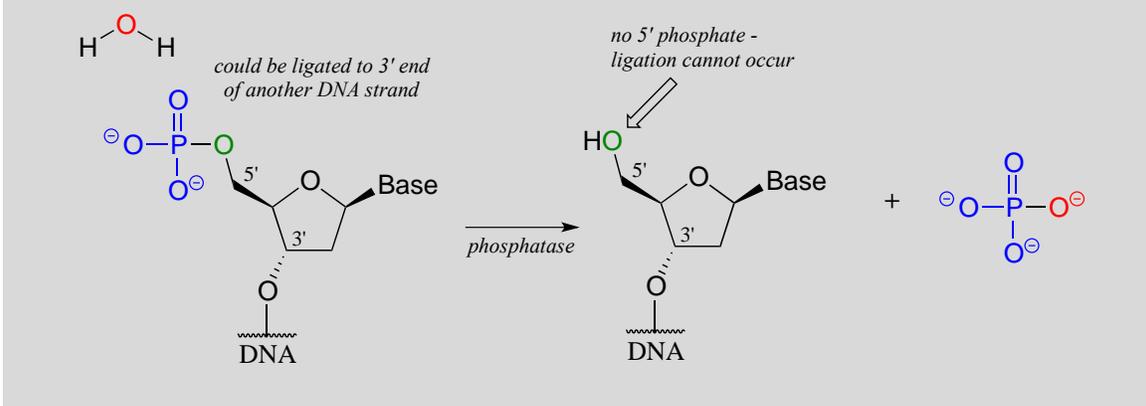


Phase 2: ligation:



One more enzymatic tool in the genetic engineering arsenal bears mention. In some cloning procedures, a researcher may want to prevent unwanted ligation of DNA. This can be accomplished by using the enzyme **alkaline phosphatase** (EC 3.1.3.1), which catalyzes the dephosphorylation of many different organic phosphates, including 5'-phosphorylated DNA (recall that we discussed phosphatases in section 9.6).

Alkaline phosphatase reaction:

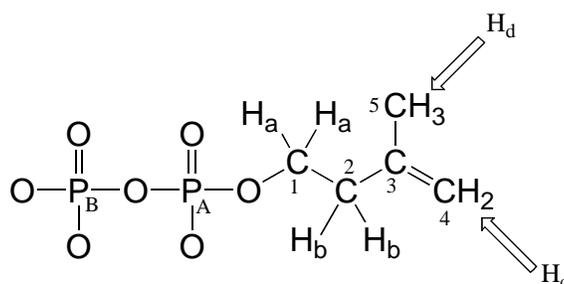


With the phosphate group removed, ligation is impossible - there is no way to make a new phosphodiester bond without a 5' phosphate group!

Section 9.9: NMR of phosphorylated compounds

Because so many biological molecules contain phosphoryl groups, it is worthwhile to look at how scientists use NMR to determine the structure of these molecules. Recall from section 5.1 that ^{31}P , the most abundant isotope of phosphorus, is NMR active: it can be directly observed by ^{31}P -NMR, and indirectly observed in ^1H -NMR and ^{13}C -NMR through its spin-coupling interactions with neighboring protons and carbons, respectively.

Consider the case of isopentenyl diphosphate, the building block molecule used by cells to make 'isoprenoid' compounds such as cholesterol (in many animals), or β -carotene (in some plants). NMR spectra of this molecule were taken in a D_2O solvent, buffered with ND_4OD (the deuterium equivalent of aqueous ammonium hydroxide, NH_4OH) (*J. Org. Chem.* **1986**, *51*, 4768). In our discussion, carbon atoms are specified with numbers, protons with lower case letters, and phosphorus atoms with upper case letters.



First, let's look at the proton spectrum:

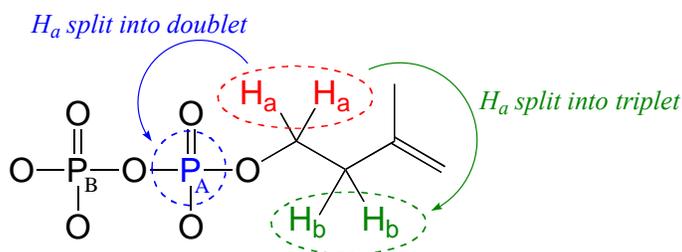
^1H -NMR

H_a : 4.05 ppm (td); $^3J_{\text{H}_a-\text{H}_b} = 6.6$ Hz; $^3J_{\text{H}_a-\text{P}_A} = 3.3$ Hz.

H_b : 2.39 ppm (t) $^3J_{\text{H}_a-\text{H}_b} = 6.6$ Hz

H_c : 4.86 ppm (s)

H_d : 1.77 ppm (s)



The signals for H_b , H_c , and H_d look like we would expect from our discussion in chapter 5, with the exception of H_c which you will be invited to discuss in the exercise below. Why, though, is the signal for H_a split into a *triplet of doublets* (td)? First of all, as expected, the two neighboring H_b protons split the H_a signal into a triplet, with $^3J_{\text{H}-\text{H}} = 6.6$ Hz. Then, **the signal is further split into doublets ($^3J_{\text{H}-\text{P}} = 3.3$ Hz) by P_A , the closer of**

the two phosphorus atoms. A phosphorus atom will spin-couple with protons up to three bonds away.

Exercise 9.12: The signal for the two 'H_c' protons in isopentenyl diphosphate is reported above as a singlet integrating to 2H. Are these two protons really chemically equivalent, and, according to what you know about proton NMR, should this signal really be a singlet? If not, what kind of signal(s) would you expect to see? Explain any discrepancies between what you would expect to see and the actual reported data.

Now, let's look at the ¹³C spectrum of IPP:

¹³C-NMR (proton-decoupled)

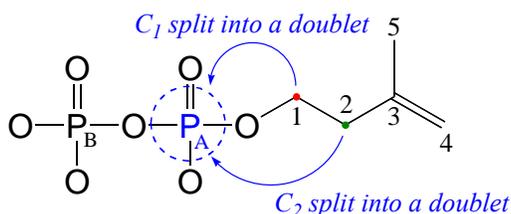
C₁: 40.7 ppm (d); ²J_{C₁-P_A} = 7.2 Hz

C₂: 67.0 ppm (d); ³J_{C₂-P_A} = 4.0 Hz

C₃: 147.4 ppm

C₄: 114.6 ppm

C₅: 24.5 ppm

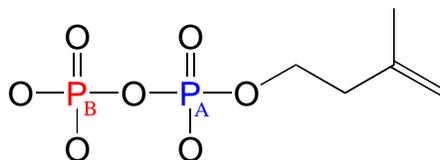


Notice that **the signals for both C₁ and C₂ are split into doublets by the magnetic field of P_A.** Phosphorus atoms will spin-couple with ¹³C nuclei up to three bonds away. Notice also that the 2-bond coupling between C₁ and P_A is larger than the 3-bond coupling between C₂ and P_A (7.2 Hz vs. 4.0 Hz). Finally, notice that we do *not* observe 4-bond C-P coupling: C₃ is not spin-coupled to P_A, and P_B is not coupled to any of the ¹³C or ¹H nuclei on the molecule.

Remember that when processing a typical ¹³C-NMR spectrum, we electronically 'turn off' spin coupling between carbons and neighboring protons in order to simplify the spectrum (this is referred to as 'proton decoupling'). Proton decoupling does **not** turn off C-P spin coupling.

Because ³¹P is NMR-active, we can also, with an NMR spectrophotometer equipped with a phosphorus probe, *directly* observe the phosphorus NMR signals, just as we can directly observe the signals from protons and ¹³C nuclei. On an NMR instrument where protons resonate at 300 MHz and ¹³C nuclei resonate at 75 MHz, phosphorus resonates at 32 MHz. In ³¹P-NMR experiments, the reference standard used to determine the 0 ppm point is usually phosphoric acid (tetramethylsilane, the standard 0 ppm point for ¹H- and ¹³C-NMR, doesn't have a phosphorus atom!). The ³¹P-NMR spectrum of isopentenyl

diphosphate has, as expected, two peaks, each of which is *upfield* of the phosphoric acid standard (negative chemical shifts!) and split into a doublet ($^2J_{P-P} = 20$ Hz) due to 2-bond coupling between the two phosphorus nuclei.



P_A : (-)11.03 ppm (d, $^2J_{P-P} = 20$ Hz)

P_B : (-)7.23 ppm (d, $^2J_{P-P} = 20$ Hz)

Notice that although the C_1 and C_2 signals were split by P_A in our ^{13}C -NMR spectrum, in the ^{31}P -NMR spectrum the converse is not true: the P_A signal is *not* split by C_1 or C_2 . Both of these carbons are NMR-inactive ^{12}C isotope in 99 out of 100 molecules. In addition, P-H splitting is not observed in this ^{31}P spectrum, because proton decoupling is in effect.

Key concepts to review

All of the reactions detailed in this chapter involved the transfer of a phosphate group - usually a phosphate, diphosphate, or AMP group - from one molecule (the donor) to another (the acceptor). Your learning goal for this chapter is to recognize and understand what is happening in these phosphate group transfer reactions, and to gain a basic understanding of the chemistry of phosphate and other phosphate groups.

Be sure that you can identify and provide examples of the following terms:

inorganic phosphate	organic triphosphate
inorganic phosphate	phosphate (mono)ester
inorganic pyrophosphate	phosphate diester
organic (mono)phosphate	phosphate anhydride
organic diphosphate	bridging/non-bridging oxygen

Also, make sure that you can recognize and use appropriately the various abbreviations introduced in this chapter:

P_i	PP_i	R-OP
R-OPP	R-OAMP	ATP
ADP	AMP	

. . . in addition to the various structural abbreviations for adenosine mono-, di-, and triphosphate.

You should know the approximate pK_a values for phosphoric acid and an organic monophosphate, and be able to state the approximate net charge (to the nearest 0.5 charge unit) of these species in buffers of different pH levels.

You should be able to describe, in words and pictures, the tetrahedral sp^3d bonding picture for the phosphorus atom of a phosphate group. *Even though the geometry is not always shown in every drawing, always keep in mind that the phosphate group is tetrahedral.*

You should be able to draw resonance contributors for different phosphate groups, identify major versus minor contributors, and explain why some are major and some are minor. *Remember - charges are shared between non-bridging oxygens, even if they are not drawn that way!*

Absolutely critical to your success with this chapter is being able to picture and illustrate the mechanistic pattern which we refer to as a phosphate group transfer.

Though not usually included in reaction illustrations, always remember that charge-charge interactions with magnesium ions and hydrogen bonds to active site amino acids both serve to increase the electrophilicity of a phosphorus atom in donor compounds such as ATP.

You should understand the distinctions between the three mechanistic models for phosphate transfer reactions - concerted, addition-elimination, and elimination-addition - and know that the concerted model probably most closely describes biochemical reactions.

You should be able to identify the apical and equatorial positions in the pentavalent transition state of a phosphate transfer reaction, and recognize that the reaction results in inversion at the phosphorus center.

You should be able to identify the α , β , and γ phosphate groups of ATP, as well as the ribose and adenosine parts of the molecule.

You should be able to explain how ATP acts as a phosphate group donor, and why such reactions are thermodynamically favorable.

You should be able to draw a curved-arrow mechanism for reactions in which ATP acts as a phosphate group donor in the phosphorylation and/or diphosphorylation of an alcohol. You should be able to predict the result of nucleophilic attack at the α , β , or γ phosphates of ATP.

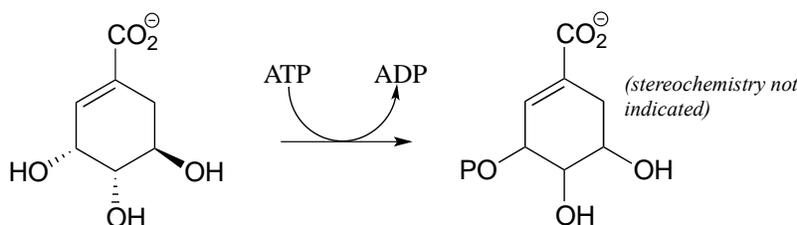
In general, you should be able to propose a likely mechanism for any phosphate transfer reaction, given the starting compounds and products.

Given information about the existence of a covalently linked enzyme-substrate complex in an enzyme mechanism, you should be able to propose a likely mechanism that accounts for such an intermediate. For example, after being told that the phosphotyrosine phosphatase reaction involves a phosphocysteine intermediate, you should be able to propose a mechanism.

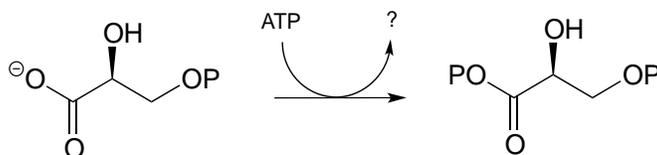
Problems

*In all of the problems that follow, feel free to use appropriate abbreviations when drawing structures. However, always be sure **not** to abbreviate regions of a structure which are directly involved in bond-breaking or bond-forming events.*

P9.1: Draw a likely mechanism for reaction catalyzed by shikimate kinase (EC 2.7.1.71) in the aromatic amino acid biosynthesis pathway). Stereochemistry of the product is not indicated in the figure below - in your mechanism, show the stereochemistry of the product, and explain how you are able to predict it from your knowledge of kinase reactions.

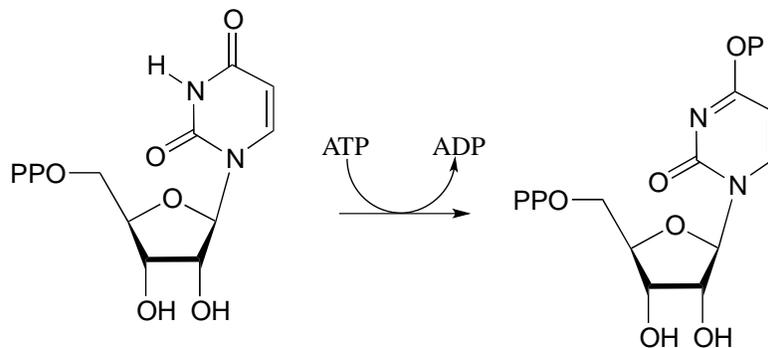


P9.2: Draw a likely mechanism for the following reaction (EC 2.7.2.3) in the gluconeogenesis pathway, and predict what compound is indicated by the question mark.

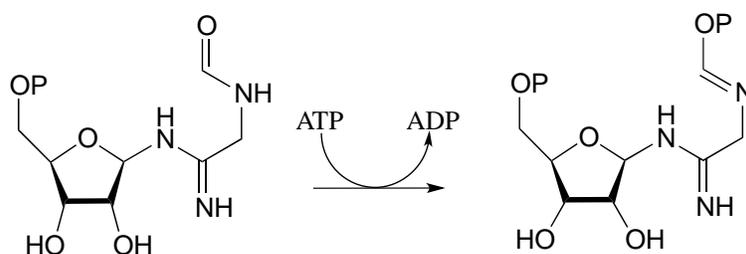


P9.3:

a) Draw a likely mechanism for the following reaction (EC 6.3.4.2) from ribonucleotide biosynthesis. Hint: what is the nucleophilic group? How could the enzyme increase its nucleophilicity?

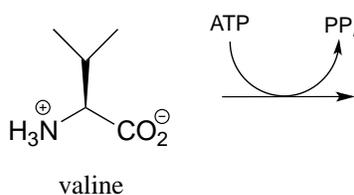


b) Draw a mechanism for the following reaction, also from ribonucleotide biosynthesis (EC 6.3.3.1):

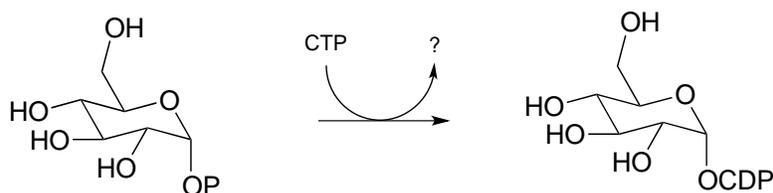


P9.4:

a) The carboxylate group on the amino acid valine is activated in an early step in the biosynthesis of the antibiotic penicillin. Predict the product of this reaction, and draw the likely intermediate of the phosphate group transfer reaction.



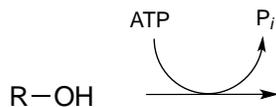
P9.5: The reaction below is an early step in the synthesis of tyvelose, a sugar found on the surface of some pathogenic bacteria. Notice that CTP plays the role of the phosphate group donor in this case, rather than ATP.



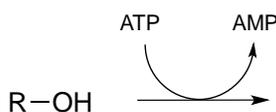
Draw a mechanism for the reaction, and indicate the second product that is released by the enzyme. (*J. Biol. Chem.* **2005**, *280*, 10774)

P9.6: Draw the likely product of the following hypothetical phosphate group transfer reactions. Specify which phosphate group of ATP is the electrophile in each case.

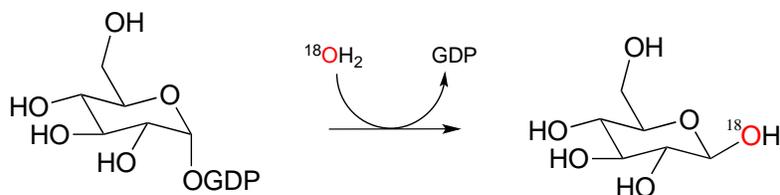
a)



b)



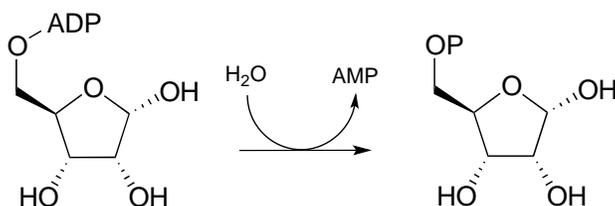
P9.7: The figure below illustrates an experiment in which a reaction catalyzed by an *E. coli* enzyme was run in isotopically labeled water.



a) The researchers concluded that the reaction was *not* a phosphate group transfer. Explain their reasoning.

b) Draw the products that would be expected if the reaction actually did proceed by a phosphate transfer mechanism (be sure to show stereochemistry and the location of the ^{18}O atom).

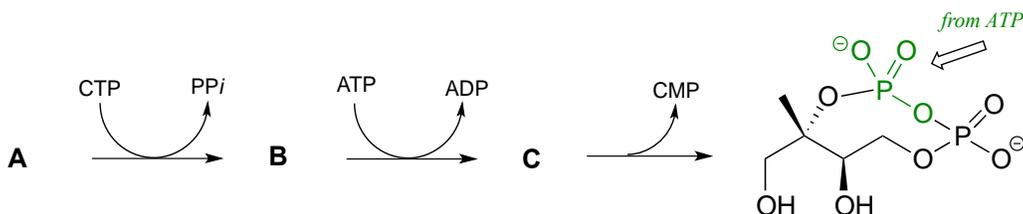
P9.8: The reaction below proceeds with a *direct* attack by a water molecule on the substrate, but the hydrolysis could be expected to proceed through two possible mechanisms. Draw two possible mechanisms for the reaction run in H_2^{18}O . Trace the progress of the ^{18}O 'label' throughout each mechanism to see where it ends up: this should indicate to you how the two mechanisms could be (and in fact were!) distinguished experimentally.



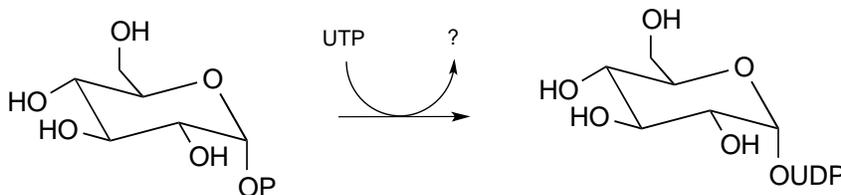
P9.9: Glucose-6-phosphate is dephosphorylated to glucose in the last step of the gluconeogenesis pathway (EC 5.3.1.9). The reaction is not a direct hydrolysis: like the phosphotyrosine phosphatase reaction we saw in this chapter it involves formation of a phosphoenzyme intermediate, but in this case the enzyme residue acting as the initial phosphate acceptor is an active site *histidine* rather than an aspartate. Given this information, propose a likely mechanism for the reaction.

P9.10: (This question assumes a basic knowledge of DNA structure and the idea of *supercoiling*). DNA topoisomerase enzymes catalyze the temporary 'nicking' of one strand of double-stranded DNA, which allows supercoiled DNA to 'unwind' before the nicked strand is re-ligated. During the unwinding process, the 5' end of the nicked strand is transferred to a tyrosine in the enzyme's active site, effectively holding it in place while the 3' end rotates. Overall, the stereochemical configuration of the bridging phosphate is retained. Propose a likely mechanism for this nicking and re-ligating process.

P9.11: Pictured below is a series of phosphate group transfer steps in the early part of isoprenoid biosynthesis in bacteria. With the knowledge that the atoms in green are derived from ATP, predict the structures of compounds A, B and C.

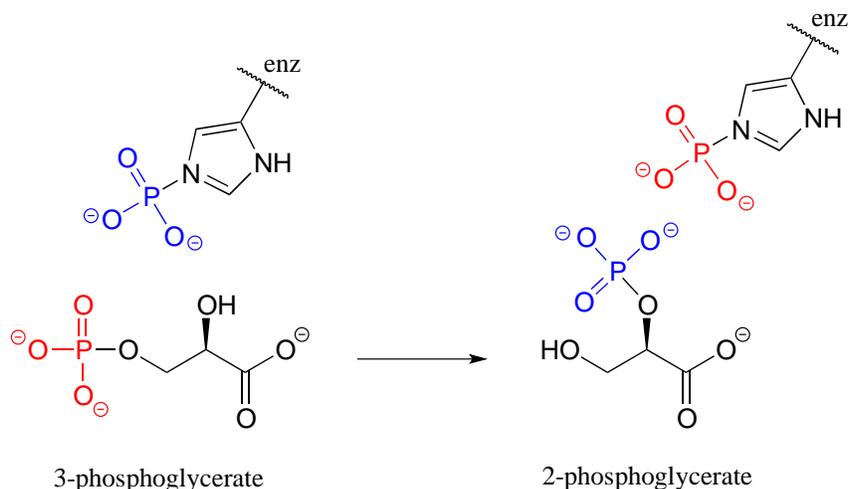


P9.12: The reaction below shows the synthesis of glucose-UDP, an important intermediate in carbohydrate biosynthesis. Notice that UTP (instead of ATP) is the phosphate donor. Identify the by-product denoted below by a question mark.

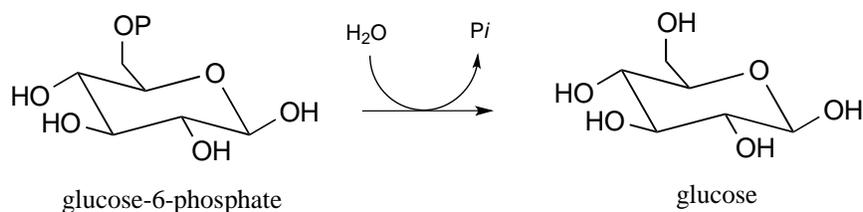


P9.13: Isomerization of 3-phosphoglycerate to 2-phosphoglycerate (EC 5.4.2.1, a reaction in glycolysis) has been shown to occur with the participation of a phosphohistidine residue in the enzyme's active site. The two phosphate groups are

distinguished in the figure below by color. With this information, propose a mechanism for the reaction.



P9.14: The gluconeogenesis (sugar-building) pathway enzyme glucose-6-phosphatase catalyzes an *indirect* phosphate hydrolysis reaction with a phosphohistidine intermediate ('indirect hydrolysis' in this context means that a water molecule does *not* directly attack glucose-6-phosphate).



Researchers wanted to confirm that the hydrolysis in this reaction is indirect, rather than direct. It turns out that the same enzyme is also capable of catalyzing the transfer of the phosphate group from glucose-6-phosphate to the hydroxyl group on carbon #6 of another glucose molecule (instead of to water, which is the natural reaction). The enzyme-catalyzed transfer of phosphate between two glucose substrates is reversible.

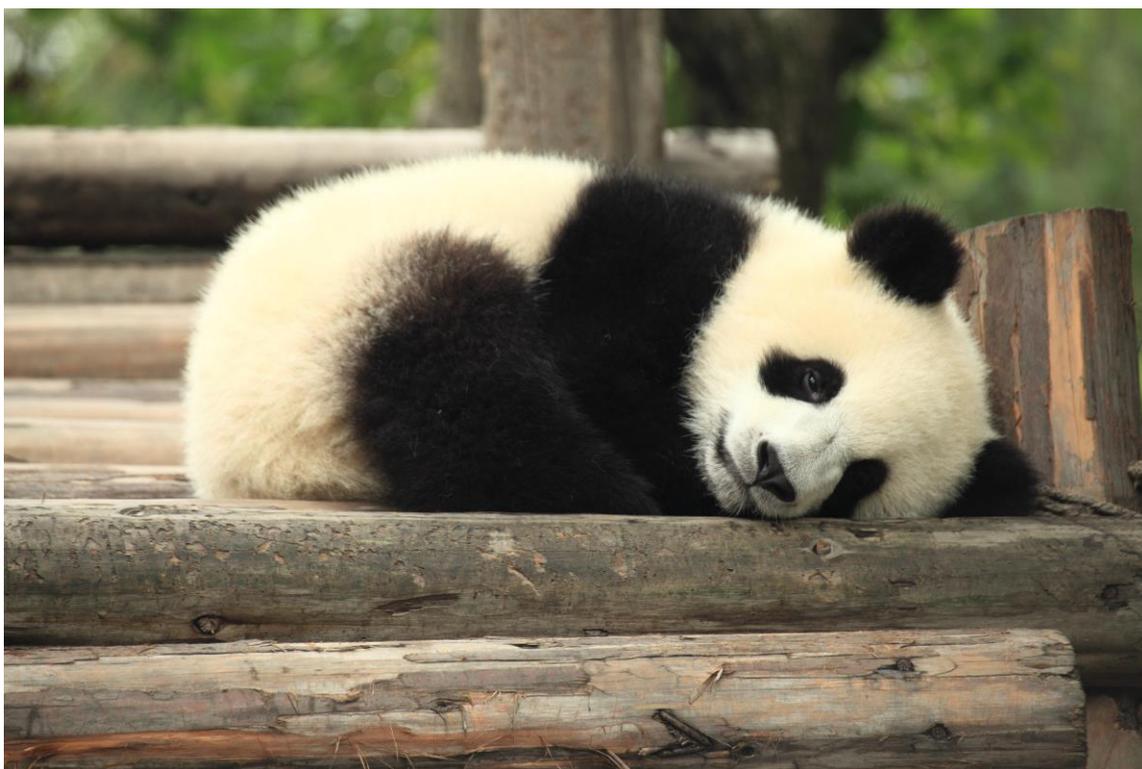
The researchers incubated the enzyme with labeled glucose-6-phosphate, in which the phosphate center was chiral (with the *R* configuration) due to the incorporation of ^{17}O and ^{18}O isotopes. They also included a high concentration of glucose in the reaction mixture, which ensured that the glucose-to-glucose transfer reaction predominated and hydrolysis (the 'natural' reaction) did not take place. After allowing the reaction to reach equilibrium, they isolated the glucose-6-phosphate and looked at the configuration of the phosphate group.

Given what you have just learned about the enzyme mechanism, predict what the researchers found in this experiment, explain your prediction, and draw the appropriate structure(s), including stereochemistry. Assume that the glucose-to-glucose mechanism is

identical to the hydrolysis mechanism, aside from the identity of the ultimate phosphate acceptor.

Chapter 10

Nucleophilic addition to carbonyl groups



(photo credit: <https://www.flickr.com/photos/gzlu/>)

Introduction

It's possible that the fuel for the car you drive thirty years from now will come from the back end of a panda. Not literally, of course – but it just might turn out that future biofuel technology will be derived in part from the stuff that workers have to clean out of the enclosure housing Ya Ya and Le Le, the two resident pandas at the Memphis Zoo in . At least, that's the hope of Dr. Ashli Brown, a biochemistry professor at Tennessee State University.

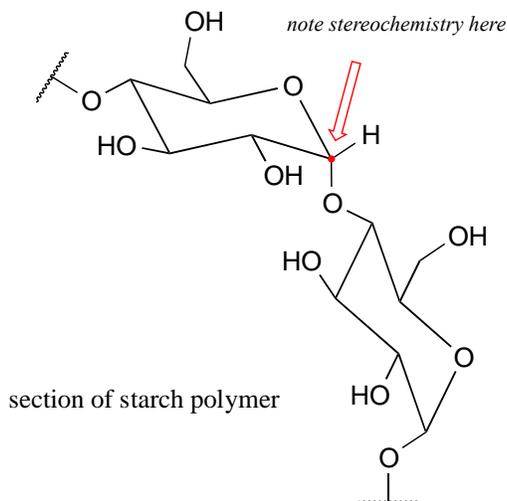
First, a little background. If you are like most people in the United States, you are already burning ethanol every time you drive: in 2012, the U.S. Department of Energy reports that over 13 million gallons of ethanol were sold at gas stations nationwide, most often as a 10% mixture along with 90% conventional gasoline. The ethanol we burn today is made by fermenting the sugars present in edible corn. The use of corn ethanol, while a significant step forward in the effort to move away from petroleum fuels and towards carbon-neutral, renewable energy sources, is far from a permanent, sustainable solution to the world's ever-increasing energy needs. Growing corn crops requires a lot of energy and expense, from running the large equipment used to plow and harvest the fields, to manufacturing and applying pesticides and fertilizers, all the way to trucking the corn to the ethanol plant. In fact, some calculation methods suggest that more energy goes into producing a gallon of corn-based ethanol than is released when the ethanol is burned.

Moreover, growing corn requires a lot of water, and takes up land which otherwise could be used for growing food, or preserved as a natural habitat. A recent study by scientists in South Dakota reported that between 2006 and 2011, a full 1.3 million acres of wetland and prairie were plowed over and converted to biofuel crop production in five midwestern states.

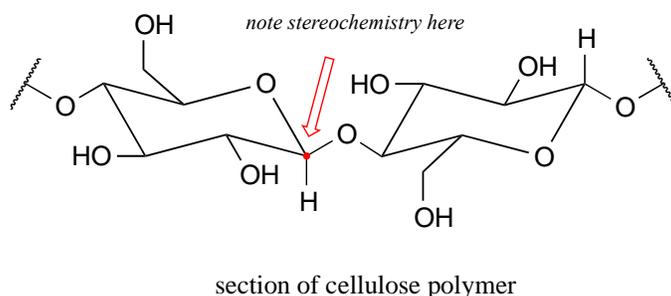
What would be much better in the long run is if we could produce ethanol or other biofuels not from resource-intensive food crops like corn, but from non-edible plant materials: grasses, trees, and agricultural byproducts such as the cobs and stalks from corn plants. Switchgrass, for example, is a native North American prairie grass that is thought to have high potential for biofuel production.

So if we can make ethanol from corn, couldn't we just change over to switchgrass using the same technology?

Unfortunately, it's not nearly that simple. Ethanol is made by 'feeding' glucose to living yeast cells, allowing them to break down the sugar into ethanol – a metabolic process called fermentation. Corn kernels contain sugar in the form of starch, a polysaccharide of linked glucose molecules. Enzymes called 'amylases' are used to break up the starch polymer into individual glucose molecules (as well as two-glucose units called cellobiose), which are then fermented by the yeast.



The rest of the corn plant – the stalks, leaves, and cobs – is composed in large part of another glucose polymer called cellulose.



Cellulose is a major component of plant cell walls, and is the most abundant organic compound on the planet - an enormous source of glucose for fermentation! The problem, from a renewable energy perspective, is how to get at the glucose monomers that make up the polymer. Look closely at the bond connecting two glucose monomers in starch, and then compare it to the same bond in cellulose. They both link the same two carbons of glucose, but with opposite stereochemistry. Recall that enzymes are very sensitive to the stereochemical configuration of their substrate molecules. It should come as no surprise, then, that the amylase enzymes which are so efficient at breaking apart starch are completely ineffective at breaking apart cellulose. Other enzymes, known as cellulases, are needed for this job. These enzymes do exist in nature: just think about what happens to tree branches, leaves, and other cellulose-rich plant matter that lies on the forest floor. These slowly rot away, the cellulose broken apart by cellulase enzymes in microscopic fungi.

The key word here, though, is 'slowly'. Fungi living on the forest floor are not in any great hurry to degrade the leaves and wood around them – the cellulose is not going anywhere. Fungal cellulases are, comparatively speaking, very slow, inefficient enzymes. Herein lies the biggest challenge to the development of economically viable production

of ethanol from cellulosic sources such as switchgrass or wood. Breaking the glucose-glucose bonds in cellulose is the main bottleneck in the whole process.

This is where the pandas come in.

Pandas live primarily on a diet of bamboo, obtaining their energy from the cellulose in the plant. Like other plant-eaters such as cows, horses, and sheep, pandas do not make their own cellulase enzymes. Rather, they rely on a diverse population of symbiotic microbes inhabiting their digestive tracts to do the job of cellulose digestion for them. Unlike the microbes living the slow-paced lifestyle of the forest floor, though, the panda's microbes don't have a lot of time to spare - the food is moving through the system pretty quickly. In theory, evolutionary pressure should have resulted in panda-gut microbes with speedy cellulase enzymes, and that is what Dr. Ashli Brown at Tennessee State was hoping to find as she and her research students analyzed panda feces from the Memphis Zoo. They have had some success: at the fall, 2013 meeting of the American Chemical Society, Dr. Brown announced that her group, working in cooperation with colleagues at the University of Wisconsin, had found over forty cellulose-digesting bacteria, courtesy of Ya Ya and Le Le. The next step is to clone the cellulase-encoding genes, use the DNA to produce recombinant enzyme, and see just how fast they are.

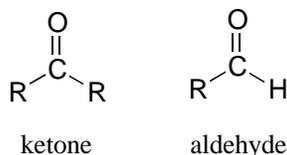
Other less cuddly and photogenic animals are also being studied with similar goals in mind. Dr. Falk Warnecke, working at the U.S. Department of Energy Joint Genome Institute in Northern California, has been investigating the microbes that live in the guts of wood-eating termites, and many other researchers around the world are interested in the symbiotic bugs which inhabit the rumen of cows and sheep.

The problematic chemical reaction catalyzed by cellulase enzymes is, in organic chemistry terminology, an 'acetal hydrolysis'. Acetals are derived from aldehydes. The reactions that occur at the carbonyl carbon of aldehydes and ketones is absolutely central to the chemistry of carbohydrates such as starch and cellulose, and it is this chemistry that is the subject of the chapter we are about to begin.

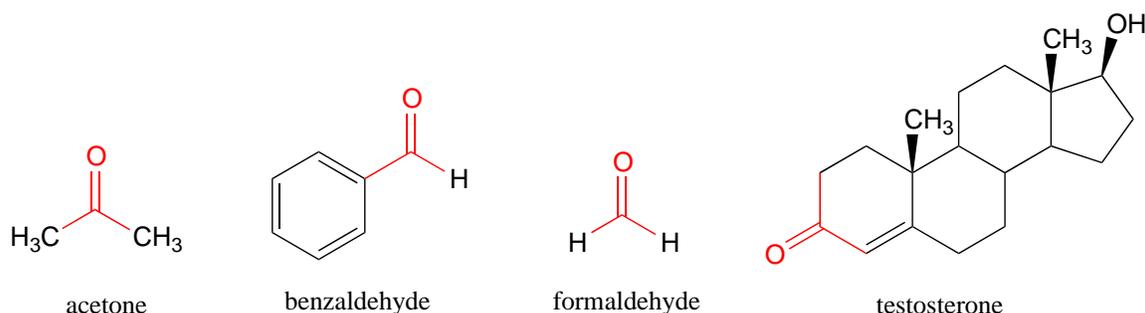
Section 10.1 Nucleophilic addition to aldehydes and ketones: an overview

10.1A: The aldehyde and ketone functional groups

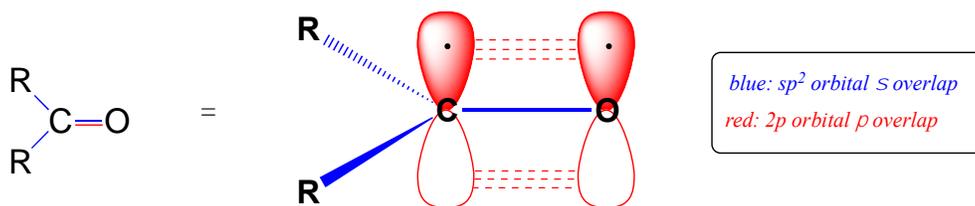
Recall from chapter 1 that the ketone functional group is made up of a carbonyl bonded to two carbons, while in an aldehyde one (or both) of the neighboring atoms is a hydrogen.



You probably are familiar with the examples shown below: acetone, the simplest ketone compound, is the solvent in nail polish remover, benzaldehyde is the flavoring in maraschino cherries, and formaldehyde (a special case in which the carbonyl carbon is bonded to hydrogens on both sides) is the nasty-smelling stuff that was used to preserve the unlucky frog that you dissected in high school biology class. The male sex hormone testosterone contains a ketone group in addition to alcohol and alkene groups.

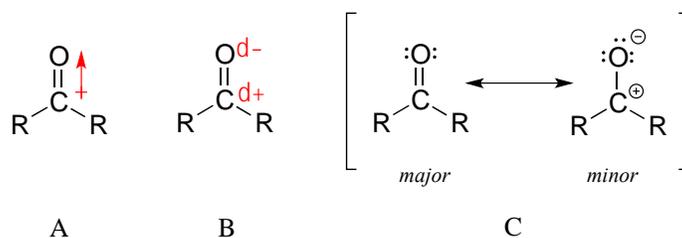


Recall from chapter 2 the bonding picture in a ketone or aldehyde: the carbonyl carbon is sp^2 hybridized, with its three trigonal planar sp^2 orbitals forming σ bonds with orbitals on the oxygen and on the two carbon or hydrogen atoms. The remaining unhybridized $2p$ orbital is perpendicular to the plane formed by the sp^2 orbitals, and forms a π bond through a side-by-side overlap with a $2p$ orbital on the oxygen. The σ and p bonds between the carbon and oxygen combine to make the C=O double bond that defines the carbonyl functionality.



10.1B: Nucleophilic addition

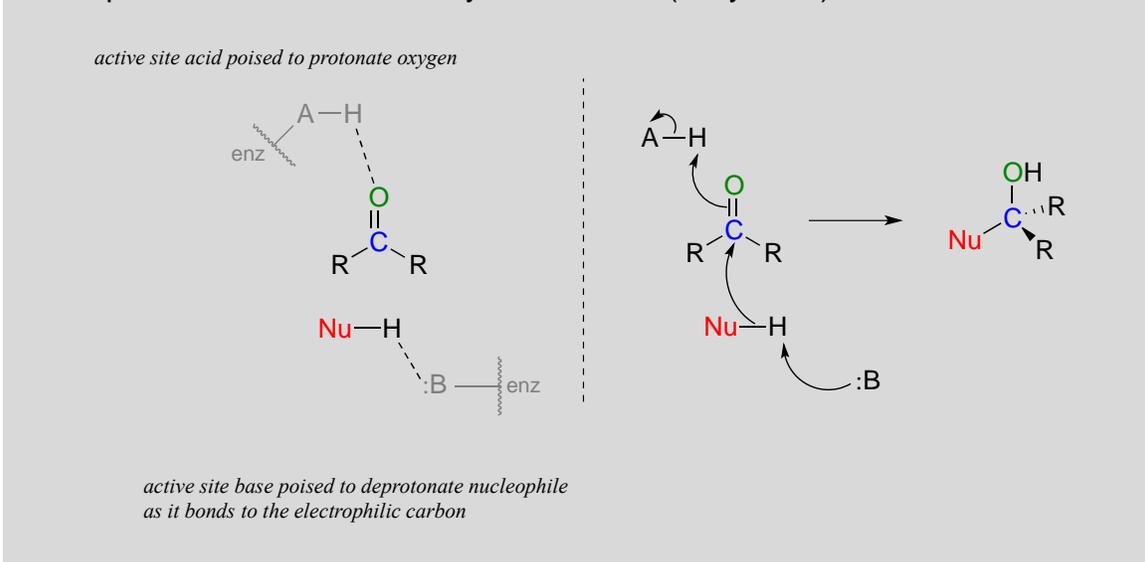
The carbon-oxygen double bond is polar: oxygen is more electronegative than carbon, so electron density is higher on the oxygen end of the bond and lower on the carbon end. Recall that bond polarity can be depicted with a dipole arrow (A in the figure below), or by showing the oxygen as bearing a partial negative charge and the carbonyl carbon a partial positive charge (B).



A third way to illustrate the carbon-oxygen dipole (C in the figure above) is to consider the two main resonance contributors: the major form, which is what you typically see drawn in Lewis structures, and a minor but very important contributor in which both electrons in the π bond are localized on the oxygen, giving it a full negative charge. The latter depiction shows the carbon with an empty $2p$ orbital and a full positive charge.

However the bond polarity is depicted, the end result is that the carbonyl carbon is electron-poor - in other words, it is an electrophile. In addition, the trigonal planar geometry means that the carbonyl group is unhindered). Thus, it is an excellent target for attack by an electron-rich nucleophilic group, a mechanistic step called **nucleophilic addition**:

Nucleophilic addition to an aldehyde or ketone (enzymatic)



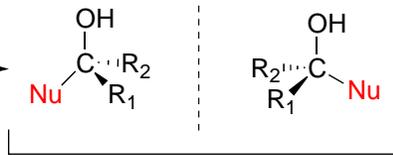
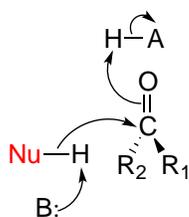
Notice the acid-base catalysis that is going on in this generalized mechanism: in the enzyme active site, a basic group is poised to deprotonate the nucleophile (thus enhancing its nucleophilicity) as begins to attack the carbonyl carbon, while at the same time an acidic proton on another active site group is poised just above the carbonyl oxygen (thus enhancing the electrophilicity of the carbon), ready to protonate the oxygen and neutralize any negative charge that builds up.

10.1C: Stereochemistry of nucleophilic addition to a carbonyl

Recall from section 3.11B that when the two groups adjacent to a carbonyl are not the same, we can distinguish between the *re* and *si* 'faces' of the planar structure.

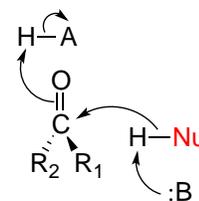
The concept of a trigonal planar group having two distinct faces comes into play when we consider the stereochemical outcome of a nucleophilic addition reaction. Notice that in the course of a carbonyl addition reaction, the hybridization of the carbonyl carbon changes from sp^2 to sp^3 , meaning that the bond geometry changes from trigonal planar to tetrahedral. If the two R groups are not equivalent, then a chiral center is created upon addition of the nucleophile. The configuration of the new chiral center depends upon which side of the carbonyl plane the nucleophile attacks from.

attack at *re* face:



enantiomers

attack at *si* face:



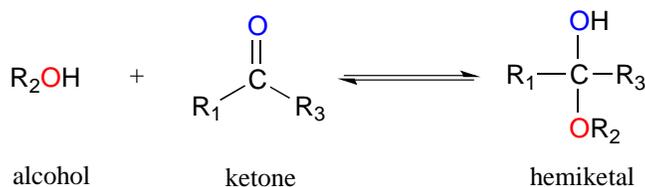
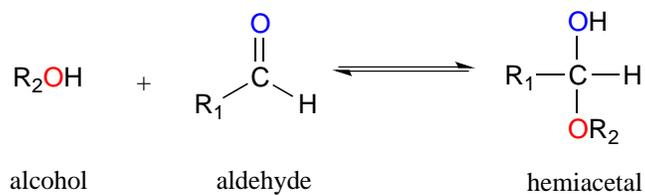
(assume R_1 is higher priority than R_2)

If the reaction is catalyzed by an enzyme, the stereochemistry of addition is (as you would expect) tightly controlled, and leads to one stereoisomer exclusively- the nucleophilic and electrophilic substrates are bound in specific positions within the active site, so that attack must occur specifically from one side and not the other. Nonenzymatic reactions of this type often result in a 50:50 mixture of stereoisomers, but it is also possible that one stereoisomer may be more abundant, depending on the structure of the reactants and the conditions under which the reaction takes place. We'll see some examples of this phenomenon soon when we look at cyclic forms of sugar molecules.

Section 10.2: Hemiacetals, hemiketals, and hydrates

10.2A: Overview

One of the most important examples of a nucleophilic addition reaction in biochemistry, and in carbohydrate chemistry in particular, is the addition of an alcohol to a ketone or aldehyde. When an alcohol adds to an aldehyde, the result is called a **hemiacetal**; when an alcohol adds to a ketone the resulting product is a **hemiketal**.



(The prefix '*hemi*' (half) is used in each term because, as we shall soon see, addition of a second alcohol nucleophile can occur, resulting in species called **acetals** and **ketals**.)

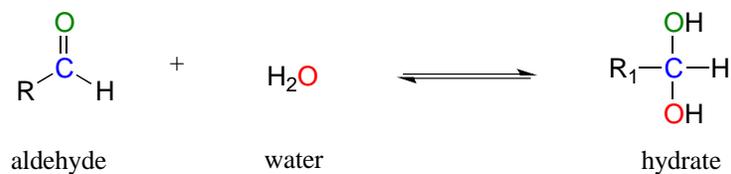
The conversion of an alcohol and aldehyde (or ketone) to a hemiacetal (or hemiketal) is a reversible process. The generalized mechanism for the process at physiological pH is shown below.

Biochemical mechanism of hemiacetal formation:



In general, hemiacetals (and hemiketals) are higher in energy than their aldehyde-alcohol components, so the equilibrium for the reaction lies to the left. As we will soon see in the context of glucose and other sugars, however, five- and six-membered *cyclic* hemiacetals are considerably lower in energy, and are favored at equilibrium: recall from chapter 3 the inherent stability of five- and six-membered rings.

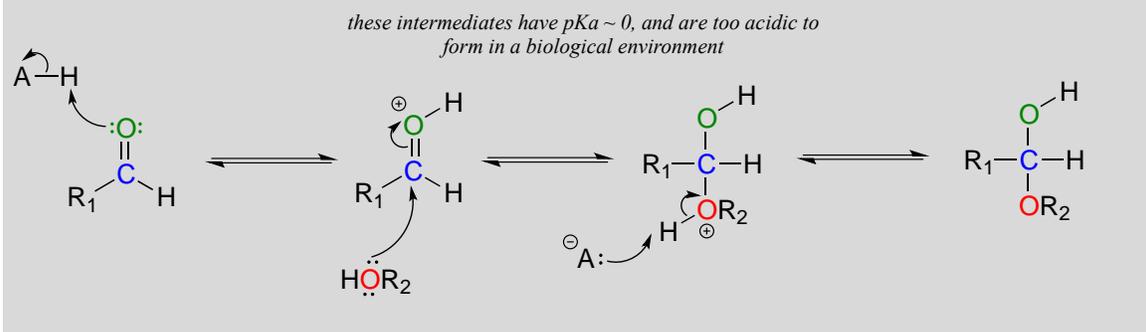
Aldehydes and ketones, when in aqueous solution, exist in equilibrium with their **hydrate** form. A hydrate forms as the result of a water molecule adding to the carbonyl carbon of the aldehyde or ketone.



Although you should be aware that aldehyde and ketone groups may exist to a considerable extent in their hydrated forms when in aqueous solution (depending upon their structure), they are usually drawn in their non-hydrated form for the sake of simplicity.

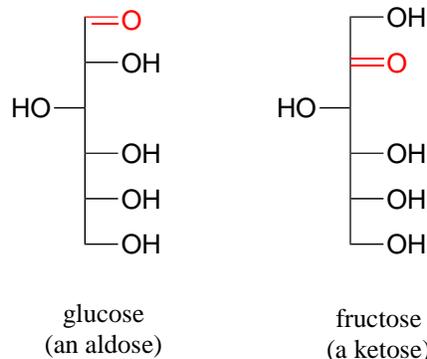
The mechanism we just saw for hemiacetal formation applies to biochemical reactions occurring at physiological pH. In the organic laboratory, however, hemiacetal and hemiketal formation usually takes place in the presence of a strong acid. The acid catalyzes the reaction by protonating the carbonyl oxygen, thus increasing the electrophilicity of the carbonyl carbon. Notice in the mechanism below that highly acidic intermediates are drawn which would be unreasonable to propose for the corresponding biochemical mechanisms occurring at physiological pH.

Acid-catalyzed hemiacetal formation (non-biological):



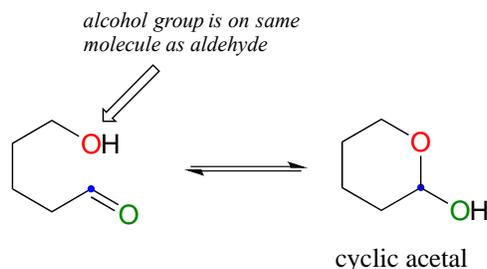
10.2B: Sugars as intramolecular hemiacetals and hemiketals

As stated above, the reactions of hemiacetals and hemiketals are central to the chemistry of carbohydrates. Recall that sugar molecules generally contain either an aldehyde or a ketone functional group, in addition to multiple alcohol groups. Aldehyde sugars are often referred to as **aldoses**; ketone sugars as **ketoses**. For example, glucose is an aldose, and fructose is a ketose - their structures are drawn below in Fischer projection:



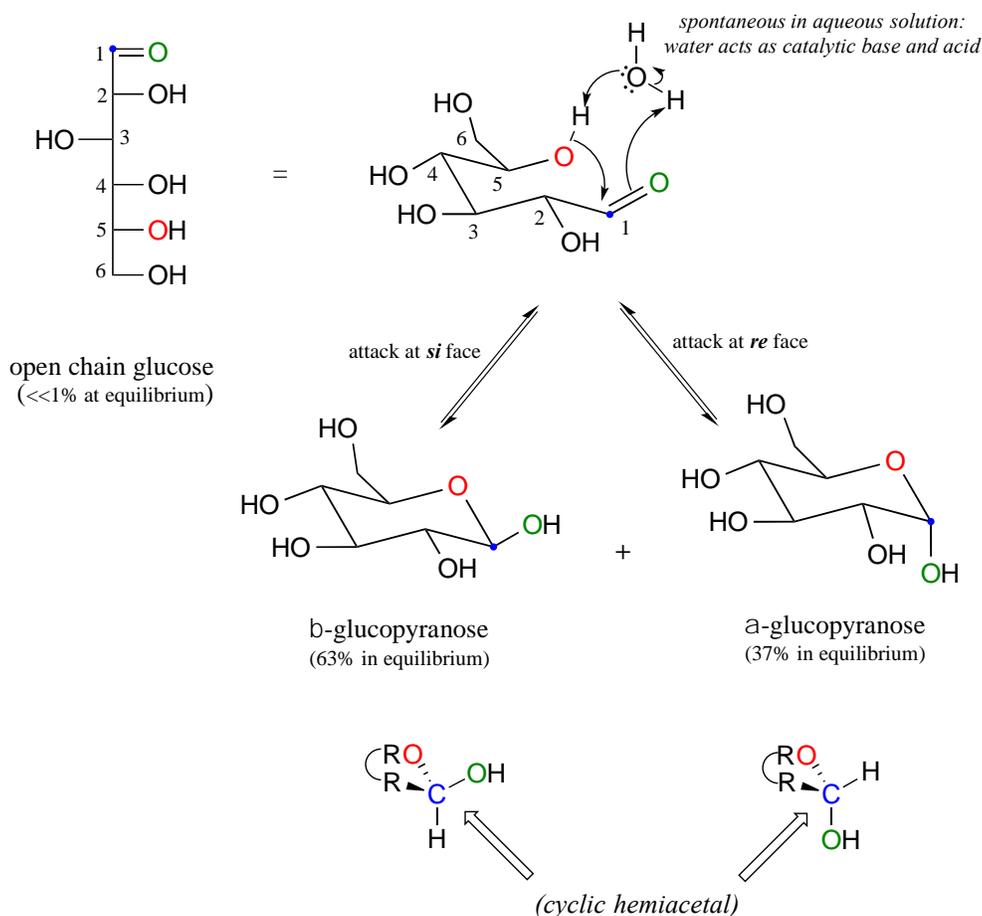
Exercise 10.1: What term describes the *relationship* between glucose and fructose (in other words, what kind of isomers are they)?

Glucose and fructose are shown above in their open-chain form. However, recall from section 1.3C that in aqueous solution, glucose, fructose, and other sugars of five or six carbons rapidly interconvert between straight-chain and cyclic forms. This occurs through the formation of *intramolecular* hemiacetals and hemiketals. This simply means that the 'R' group of the alcohol is already covalently attached to the 'R' group of the aldehyde (R₁ in our general mechanism).



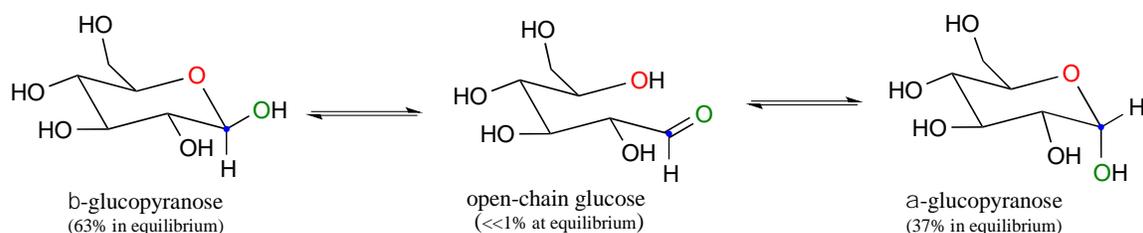
Unlike most of the biochemical reactions you will see in this text, sugar cyclization reactions *are not catalyzed by enzymes*: they occur spontaneously and reversibly in aqueous solution. For most five- and six-carbon sugars, the cyclic forms predominate in equilibrium.

The cyclic form of glucose is a six-membered ring, with an intramolecular hemiacetal formed by attack of the hydroxyl on carbon #5 to the aldehyde carbon (carbon #1, also called the **anomeric carbon** in carbohydrate terminology).



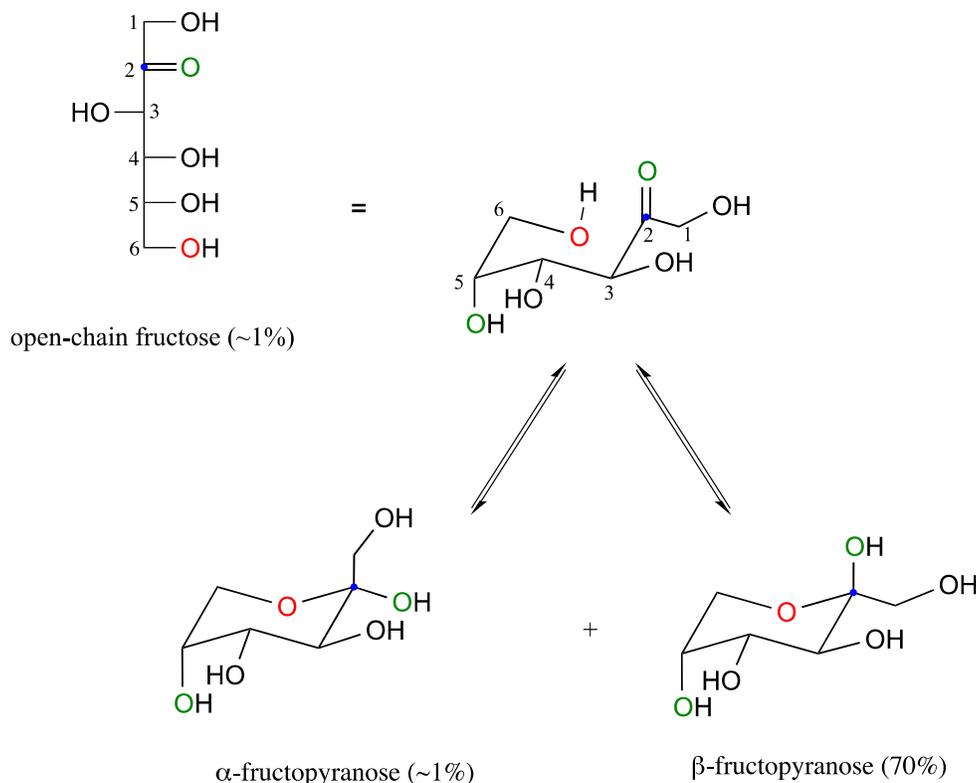
The cyclic form of glucose is called **glucopyranose**. As was discussed above, nucleophilic attack on a planar carbonyl group can occur at either face of the plane, leading to two different stereochemical outcomes - in this case, to two different diastereomers. In carbohydrate nomenclature, these two diastereomers are referred to as the α and β **anomers** of glucopyranose.

Because the formation of glucopyranose occurs spontaneously without enzyme catalysis, shouldn't equal amounts of these two anomers form? In fact, this does not happen: there is almost twice as much of one anomer than the other at equilibrium. Why is this? Remember (section 3.2) that six-membered rings exist predominantly in the chair conformation, and that the lower energy chair conformation is that in which unfavorable interactions between substituents are minimized - in most cases, this is the conformation in which larger substituents are in the *equatorial* position. In the lower-energy chair conformation of the major β anomer of glucopyranose, all of the hydroxyl groups are in the equatorial position, but in the minor α anomer one hydroxyl group is forced into the axial position. As a result, the α anomer is higher in energy, and less abundant at equilibrium.



Exercise 10.2: Draw a mechanism for the conversion of α -glucopyranose to open-chain glucose.

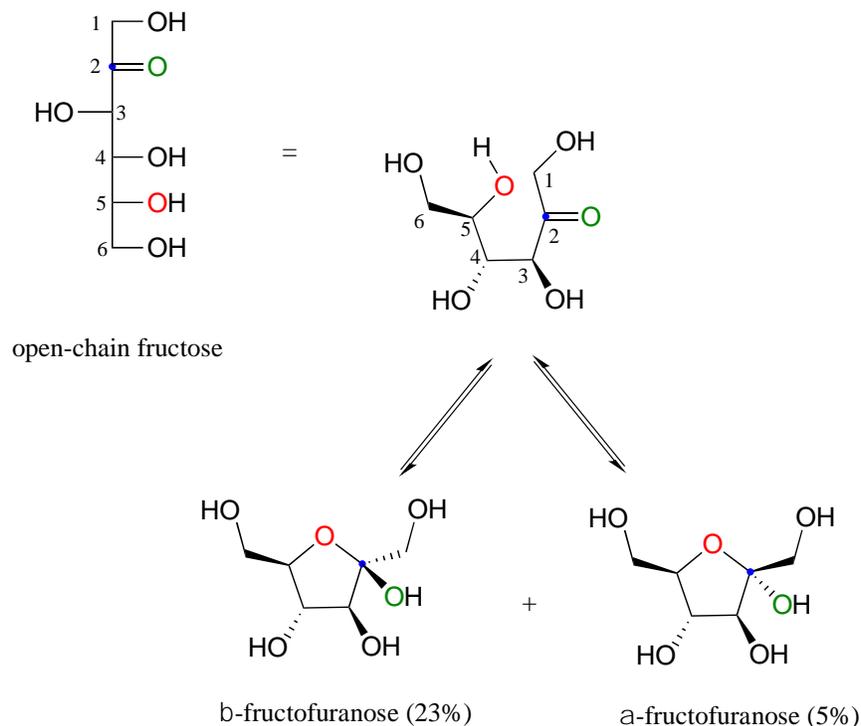
Fructose in aqueous solution forms a six-membered cyclic hemiketal called **fructopyranose** when the hydroxyl oxygen on carbon #6 attacks the ketone carbon (carbon #2, the anomeric carbon in fructose).



In this case, the β anomer is heavily favored in equilibrium by a ratio of 70:1, because in the minor α anomer the bulkier CH_2OH group occupies an axial position.

Notice in the above figure that the percentages of α and β anomers present at equilibrium do not add up to 100%. Fructose also exists in solution as a *five-membered* cyclic hemiketal, referred to in carbohydrate nomenclature as **fructofuranose**. In the formation

of fructofuranose from open-chain fructose, the hydroxyl group on the *fifth* carbon attacks the ketone.

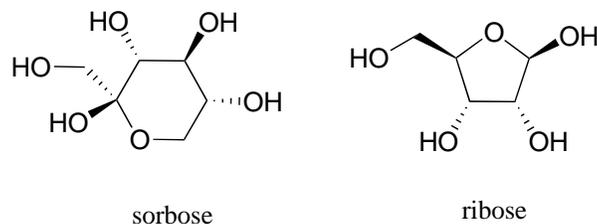


In aqueous solution, then, fructose exists as an equilibrium mixture of 70% β -fructopyranose, 23% β -fructofuranose, and smaller percentages of the open chain and cyclic α -anomers. The β -pyranose form of fructose is one of the sweetest compounds known, and is the main component of high-fructose corn syrup. The β -furanose form is much less sweet.

Although we have been looking at specific examples for glucose and fructose, other five- and six-carbon monosaccharides also exist in solution as equilibrium mixtures of open chains and cyclic hemiacetals and hemiketals. Shorter monosaccharides are unlikely to undergo analogous ring-forming reactions, however, due to the inherent instability of three and four-membered rings.

Exercise 10.3:

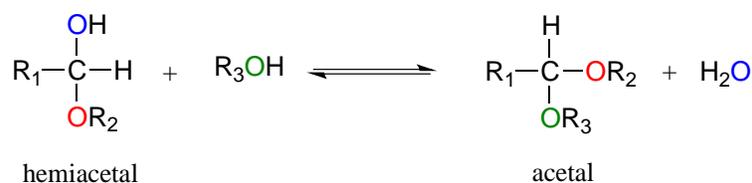
- Identify the anomeric carbon of each of the sugars shown below, and specify whether the structure shown is a hemiacetal or hemiketal.
- Draw mechanisms for cyclization of the open-chain forms to the cyclic forms shown.



Section 10.3: Acetals and ketals

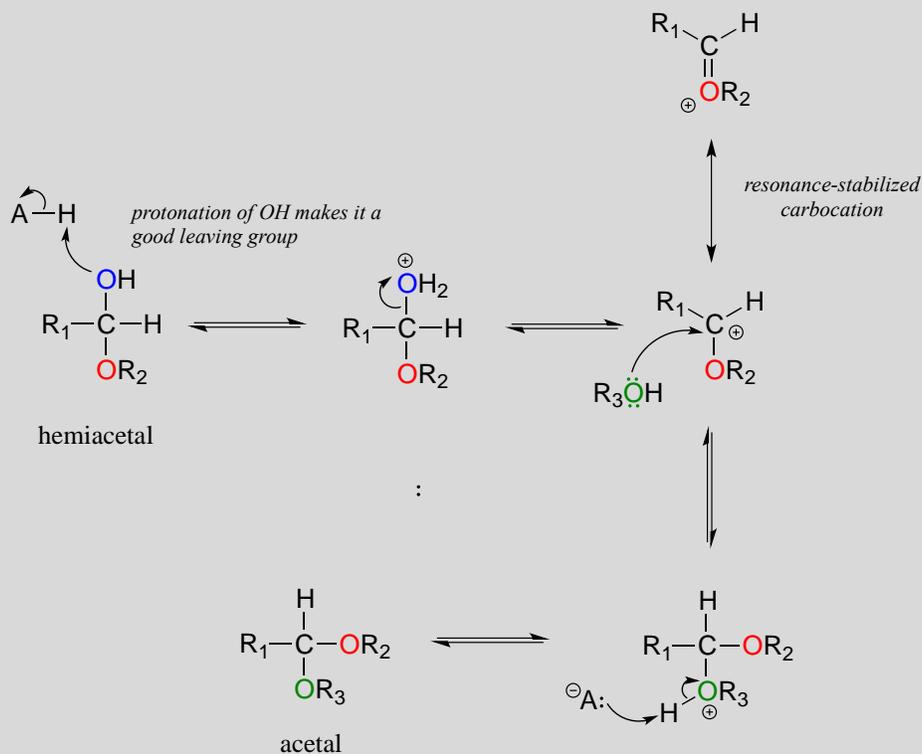
10.3A: Overview

Hemiacetals and hemiketals can react with a second alcohol nucleophile to form an acetal or ketal. The second alcohol may be the same as the first (ie. if $R_2 = R_3$ in the scheme below), or different.



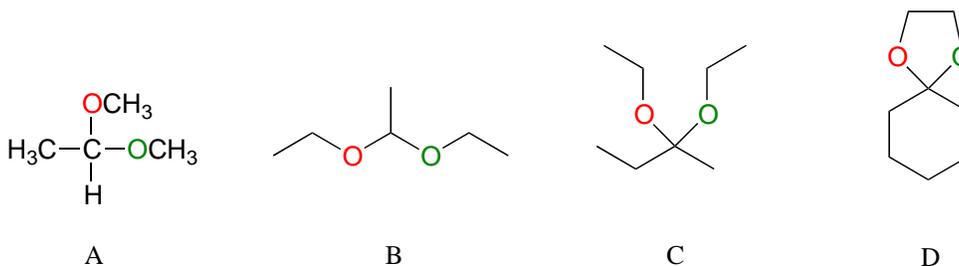
Although we focus here on biological reactions, it is instructive in this case to consider non-biological acetal-forming reactions before we look at their biochemical counterparts. In a non-enzymatic context, acetal/ketal formation - just like hemiacetal/hemiketal formation - is generally catalyzed by a strong acid.

Acid-catalyzed acetal formation (non-biological)



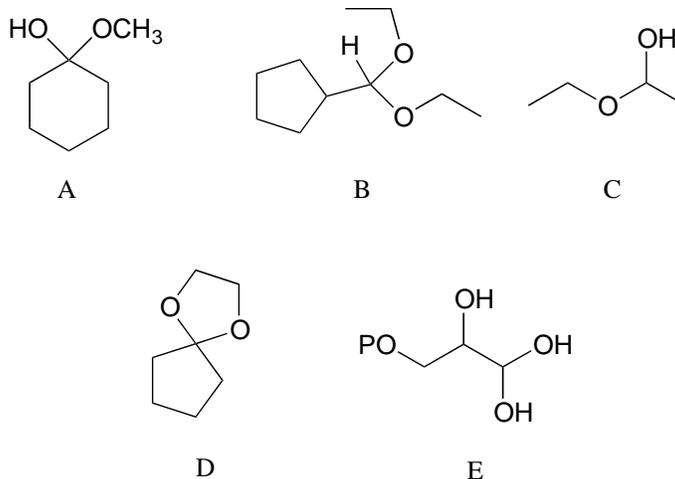
The role of the acid catalyst is to protonate the OH group of the acetal, thus making it a good leaving group (water). Notice something important here: ***the conversion of a hemiacetal to an acetal is simply an S_N1 reaction, with an alcohol nucleophile and water leaving group.*** The carbocation intermediate in this S_N1 mechanism is stabilized by resonance due to the oxygen atom already bound to the electrophilic carbon.

Below are some examples of simple, non-biological acetal and ketals.

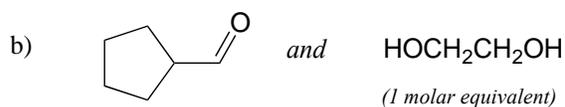
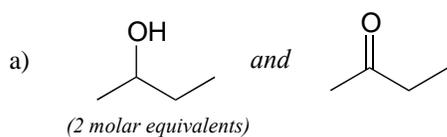


Exercise 10.4: For each acetal/ketal A-D in the figure above, specify the required aldehyde/ketone and alcohol starting materials.

Exercise 10.5: Categorize each of the following molecules as a hemiacetal, hemiketal, acetal, ketal, hydrate of an aldehyde, or hydrate of a ketone.



Exercise 10.6: Specify the acetal/ketal that would form from a reaction between the given starting compounds.

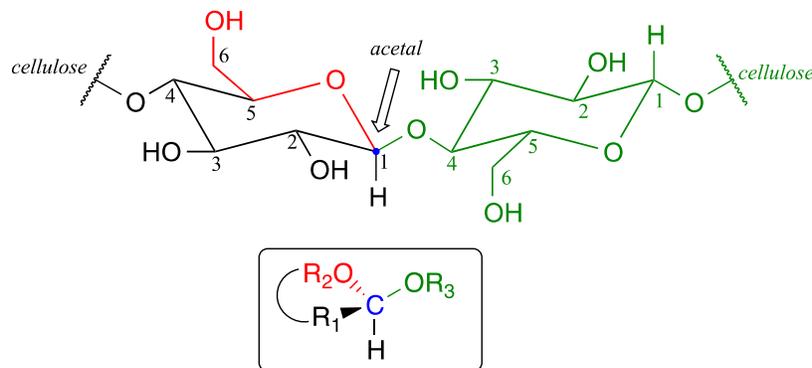


Exercise 10.7: Specify the aldehyde/ketone and alcohol combination that would be required to form the compounds in exercise 10.5.

10.3B: Glycosidic bond formation

Now, let's consider acetal formation in a biochemical context. A very important example of the acetal/ketal group in biochemistry is the glycosidic bonds which link individual

sugar monomers to form polysaccharides (see section 1.3C for a quick review). Look at the glycosidic bond between two glucose monomers in a cellulose chain:

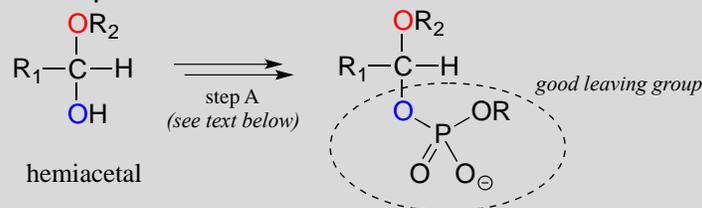


If you look carefully, you should recognize that carbon #1, the anomeric carbon on the left-side glucose monomer, is the central carbon of an acetal group. Biochemists refer to this as a β -1,4 linkage, because the stereochemistry at carbon #1 is β in the specialized carbohydrate nomenclature system, and it is linked to carbon #4 of the next glucose on the chain. The vast structural diversity of carbohydrates stems in large part from the different linkages that are possible - both in terms of which two carbons are linked, and also the stereochemistry of the linkage. You will see many more variations of glycosidic bond linkage patterns if you study carbohydrate biochemistry in greater depth.

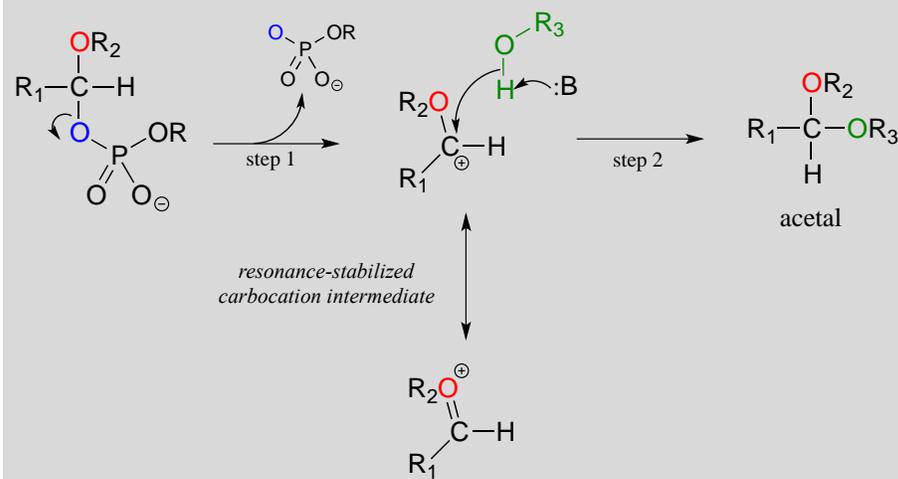
Reactions in which new glycosidic bonds are formed are catalyzed by enzymes called **glycosyltransferases**, and in organic chemistry terms these reactions represent the conversion of a hemiacetal to an acetal (remember that sugar monomers in their cyclic form are hemiacetals and hemiketals). The mechanism for glycosidic bond formation in a living cell parallels the acid-catalyzed (non-biological) acetal-forming mechanism, with an important difference: rather than being protonated, *the OH group of the hemiacetal is converted to a good leaving group by phosphorylation* (this is a pattern that we are familiar with from chapters 9 and 10). The specific identity of the activating phosphate group varies for different reactions, so it is generalized in the figure below.

Mechanism for (biochemical) acetal formation:

Hemiacetal activation phase:



acetal formation phase:



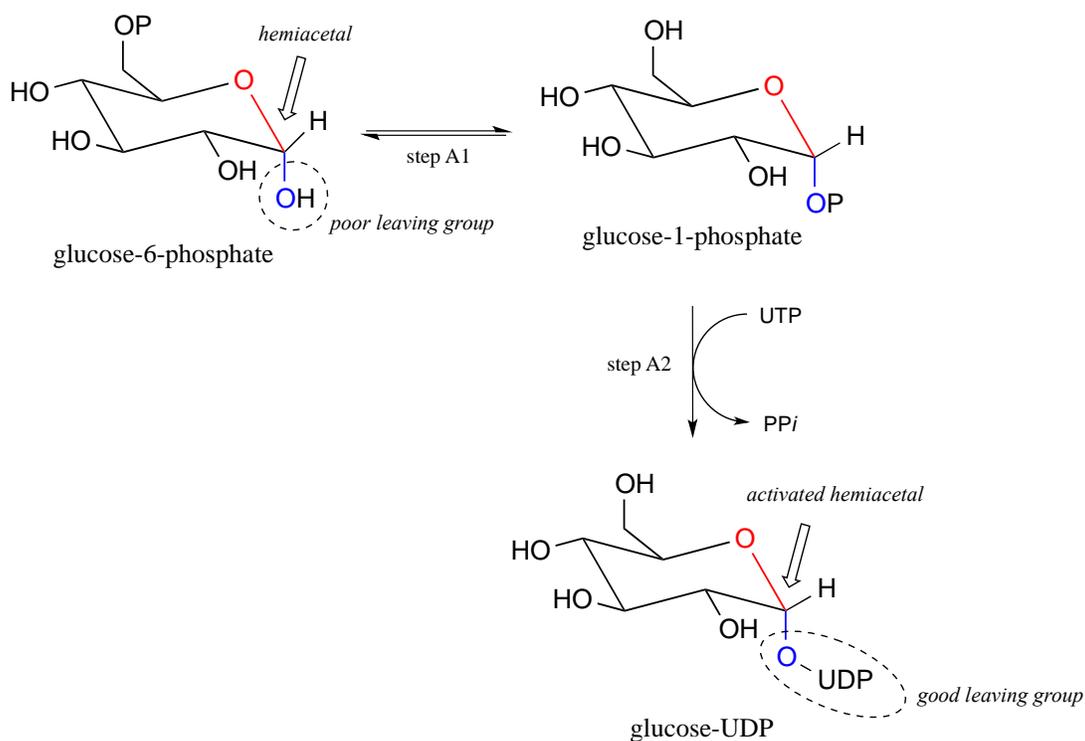
Step A (Activation phase): This phase of the reaction varies according to the particular case, but always involves phosphate group transfer steps that are familiar from chapter 9. What is most important for our present discussion, however, is simply that the hydroxyl group on the hemiacetal has been activated - i.e. made into a good leaving group - by phosphorylation.

Step 1: Now that the leaving group has been activated, it does its job and leaves, resulting in a resonance stabilized carbocation.

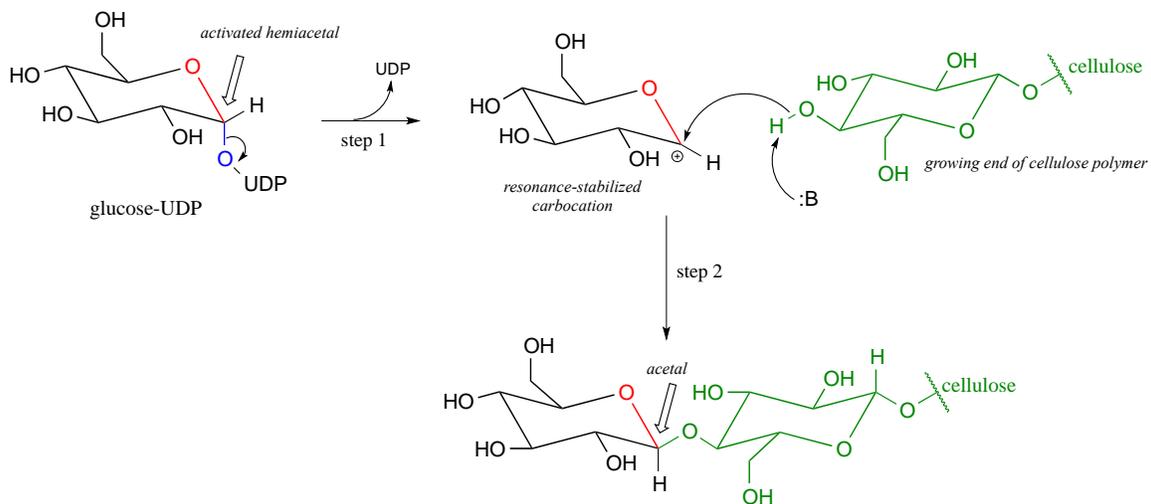
Step 2: A nucleophilic alcohol on the growing cellulose chain attacks the highly electrophilic carbocation to form an acetal. Here is where the stereochemistry of the new glycosidic bond is determined: depending on the reaction, the alcohol nucleophile could approach from either side of the planar carbocation.

To reiterate: it is important to recognize the familiar S_N1 mechanistic pattern in play here: in step A, a poor leaving group is converted into a good leaving group, in step 1 the leaving group leaves and a stabilized carbocation is left behind, and in step 2 a nucleophile attacks to form a new bond and complete the substitution process. Look back at the S_N1 reactions we saw in chapter 8 if you are having trouble making this mechanistic connection.

Now, let's look specifically at the glycosyl transferase reaction mechanism in which a new glycosidic bond is formed on a growing cellulose chain. Glucose (a hemiacetal) is first activated through two enzymatic phosphate transfer steps: step A1, a phosphate isomerization reaction with a mechanism similar to the reaction in problem P9.13, followed by a UTP-dependent step A2, for which you were invited to propose a mechanism in problem P9.12.



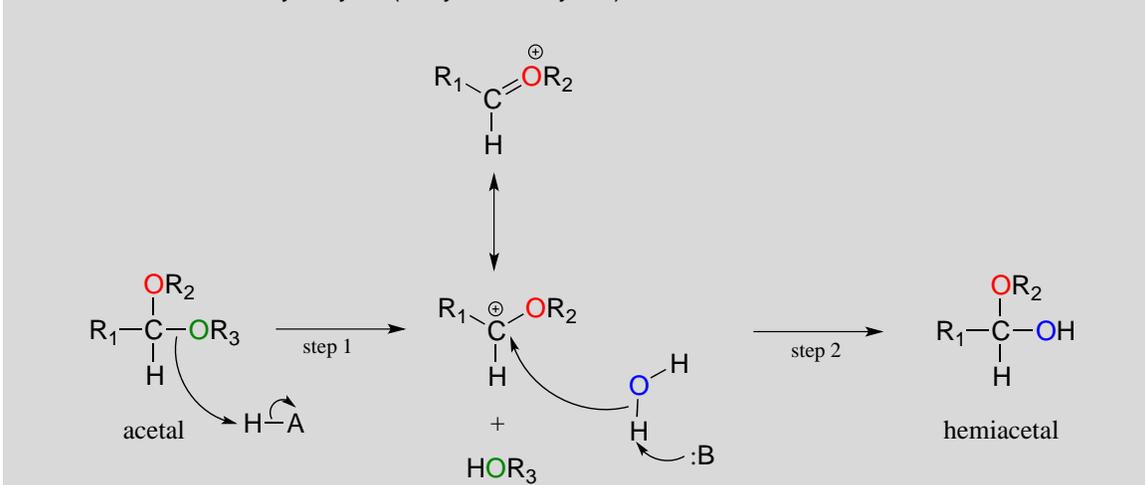
The UDP group on glucose-UDP then leaves (step 1 below), forming a resonance-stabilized carbocation intermediate. Attack by the alcohol group on the growing cellulose chain in step 2 forms the glycosidic (acetal) bond. Note the inversion of stereochemistry.



10.3C: Glycosidic bond hydrolysis

Acetals can be hydrolyzed back to hemiacetals. Notice that an acetal to hemiacetal conversion is an S_N1 -type reaction with a water nucleophile and an alcohol leaving group.

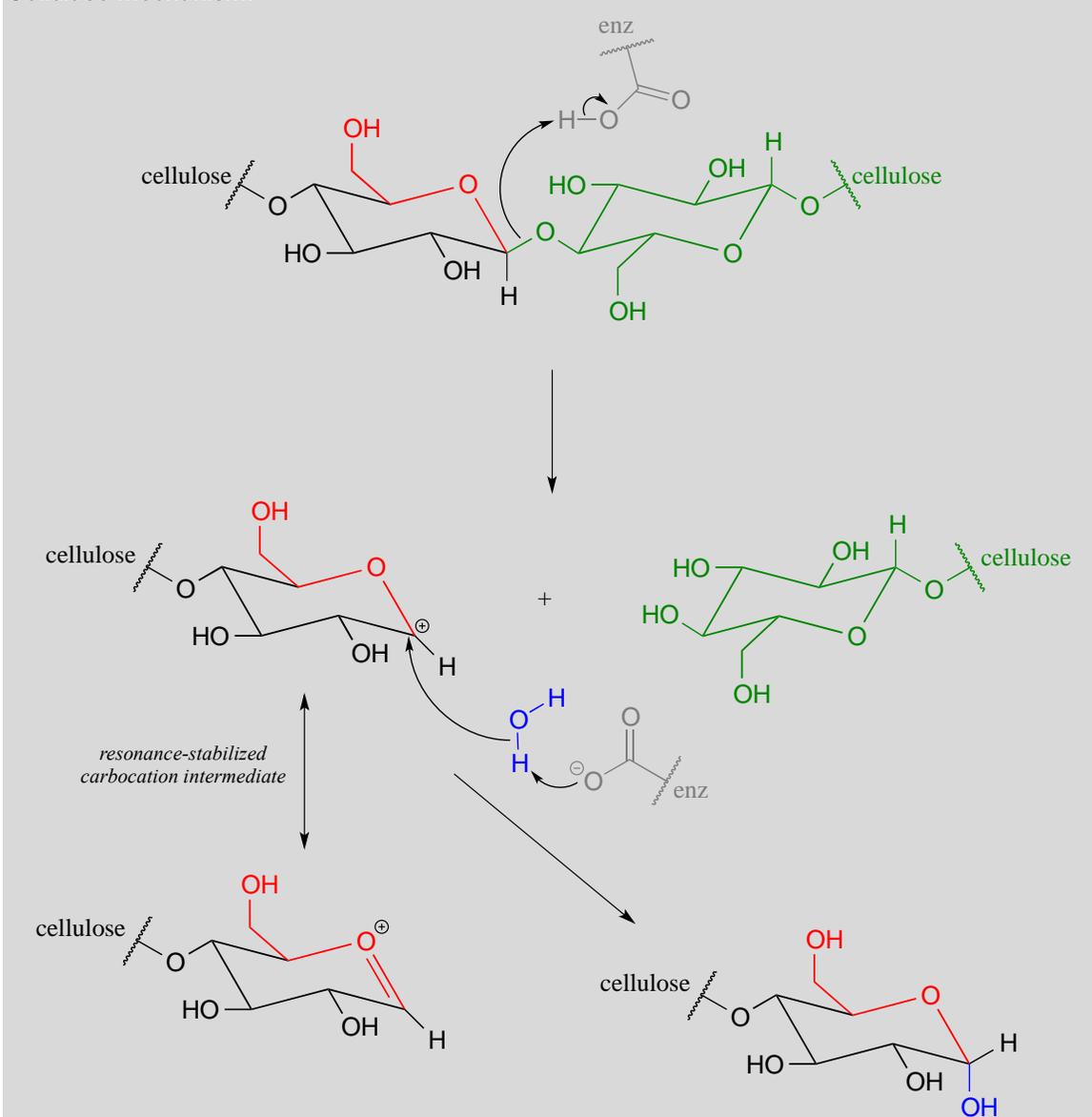
Mechanism for acetal hydrolysis (enzyme-catalyzed):



In step 1, an alcohol is protonated by a nearby acid group as it breaks away to form a resonance-stabilized carbocation intermediate. The carbocation is attacked by a nucleophilic water molecule in step 2 to form a hemiacetal.

The general mechanism above applies to reactions catalyzed by **glycosidase** enzymes, which catalyze the cleavage of glycosidic bonds in carbohydrates. In the introduction to this chapter, we learned about ongoing research in the field of cellulosic ethanol. Recall that the main bottleneck in the production of ethanol from sources such as switchgrass or wood is the **cellulase**-catalyzed step in which the glycosidic bonds in cellulose are cleaved. Cellulose-digesting microbes have several different but closely related forms of cellulase enzymes, all working in concert to cleave cellulose into smaller and smaller pieces until individual glucose molecules are free to be converted to ethanol by the fermentation process. Below is a representative mechanism for a cellulase reaction.

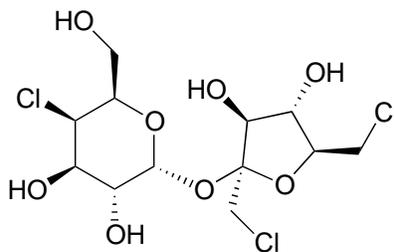
Cellulase mechanism:



The starch-digesting amylase enzymes used in the corn ethanol production process catalyze similar glycoside hydrolysis reactions, the main difference being the opposite stereochemistry at the anomeric carbon of the substrate.

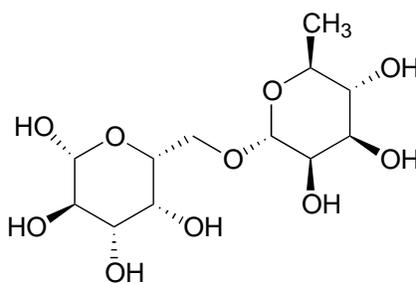
Exercise 10.8: Notice that the cellulose glycoside bond-*forming* reaction requires the cell to 'spend' a high-energy UTP molecule, but the cellulase glycoside bond-*breaking* reaction does not. Use your knowledge of chemical thermodynamics to explain this observation.

Exercise 10.9: Below is the structure of the artificial sweetener sucralose. Identify the two anomeric carbons in the disaccharide.



sucralose

Exercise 10.10: Robinose is a disaccharide found in 'Chenille Plant', a flowering shrub native to the Pacific Islands.



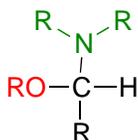
robinose

- Identify the two anomeric carbons and the glycosidic bond in robinose.
- Using the same carbon numbering system as for glucose in the earlier figure, fill in in the carbon numbers (#1 through #6) for each of the monosaccharides that make up robinose.
- Based on what you know of glycosidic bond-forming reactions in nature, propose a reasonable mechanism for the linking of the two monosaccharides, starting with the activated hemiacetal species, assuming that it is a UDP species as in the cellulose glycosidic bond-forming reaction.
- Draw the open chain form of each of the monosaccharides

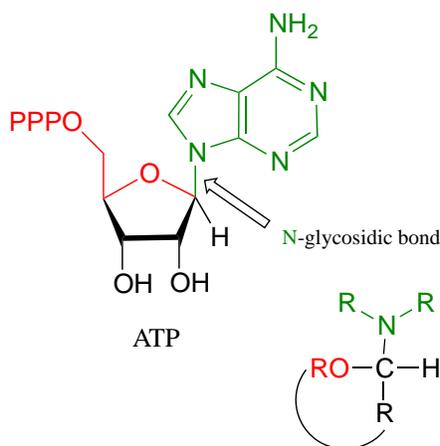
Exercise 10.11: Look again at the structures of the two-glucose fragments of cellulose and amylose shown the introduction to this chapter. A structural feature of the cellulose polymer makes it inherently more resistant to enzymatic hydrolysis compared to starch. Explain. (Hint: think about intermolecular interactions.)

Section 10.4: *N*-glycosidic bonds

We have just seen that when a second alcohol attacks a hemiacetal or hemiketal, the result is an acetal or ketal, with the glycosidic bonds in carbohydrates providing a biochemical example. But if a hemiacetal is attacked not by a second alcohol but by an amine, what results is a kind of ‘mixed acetal’ in which the anomeric carbon is bonded to one oxygen and one nitrogen.

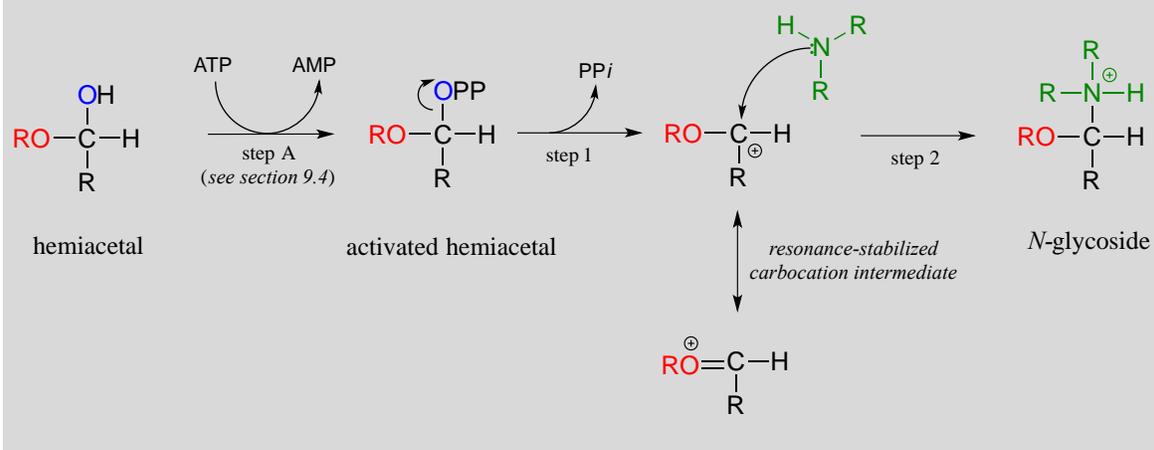


This arrangement is referred to by biochemists as an ***N*-glycosidic bond**. You may recognize these as the bonds in nucleosides and nucleotides that link the G, C, A, T, or U base to the sugar.

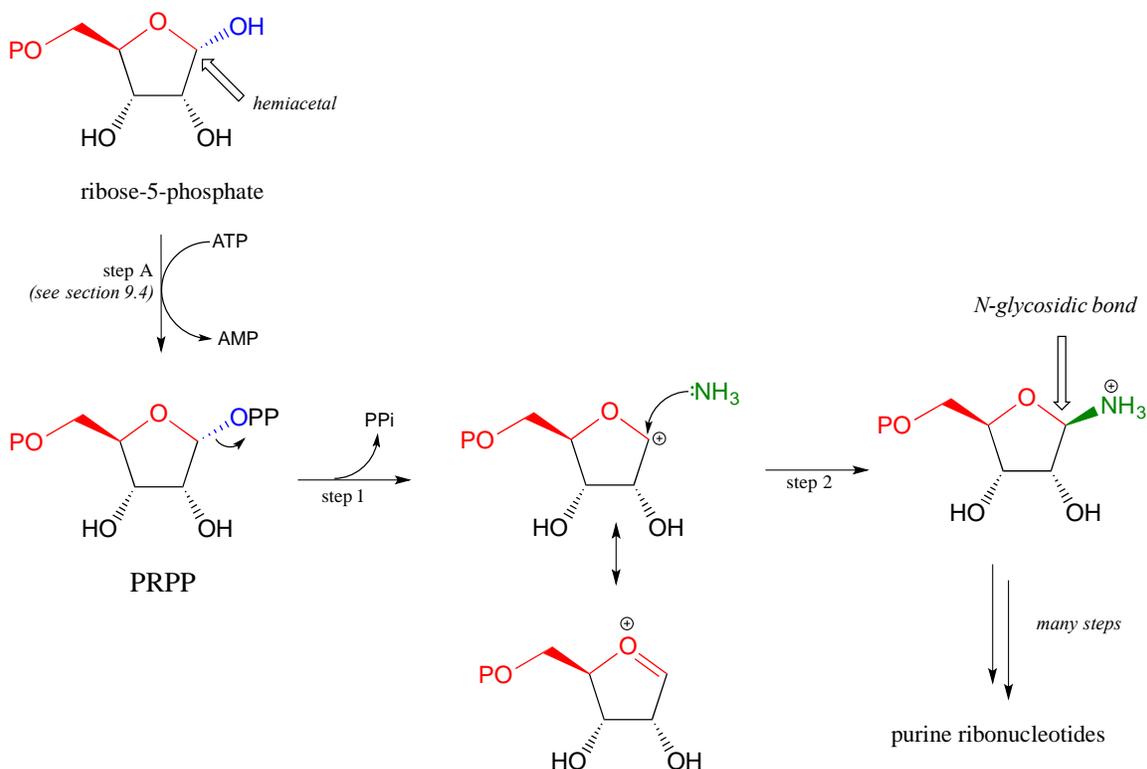


The formation of *N*-glycosidic bonds in ribonucleotides is closely analogous to the formation of glycosidic bonds in carbohydrates – again, it is an S_N1 -like process with an activated water leaving group. Typically, the hemiacetal is activated by diphosphorylation, as illustrated in step A of the general mechanism below.

Mechanism for formation of an *N*-glycosidic bond:



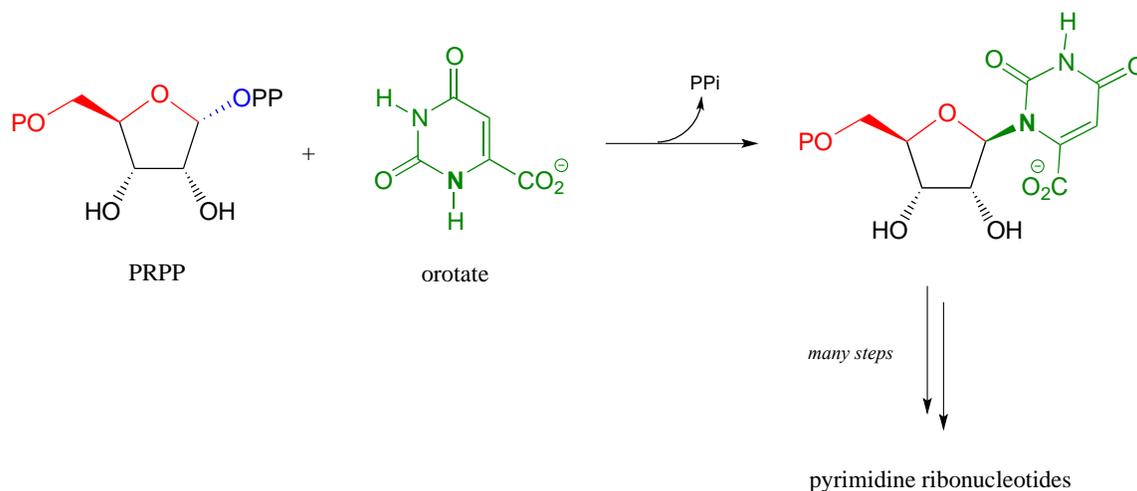
The starting point for the biosynthesis of purine (G and A) ribonucleotides is a five-carbon sugar called ribose-5-phosphate, which in solution takes the form of a cyclic hemiacetal. The critical *N*-glycosidic bond is established through substitution of NH₃ for OH at the anomeric carbon of the ribose. The anomeric OH group is first activated (step A below) to form an activated intermediate called phosphoribosylpyrophosphate (PRPP). The inorganic pyrophosphate then leaves to generate a resonance-stabilized carbocation (step 1) which is attacked by a nucleophilic ammonia in step 2 to establish the *N*-glycosidic bond.



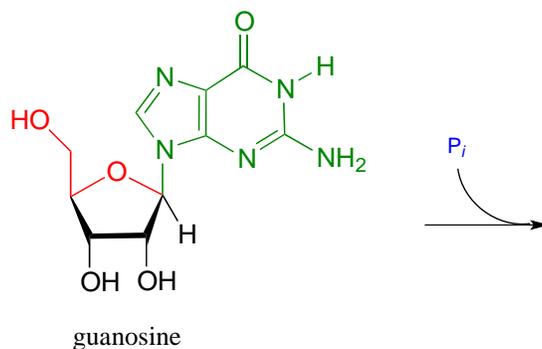
With the *N*-glycosidic bond in place, the rest of the purine base is assembled piece by piece by other biosynthetic enzymes.

(The mechanism above should look familiar - we saw step A in chapter 9 as an example of alcohol diphosphorylation, and steps 1 and 2 in chapter 8 as an example of a biochemical $\text{S}_{\text{N}}1$ reaction).

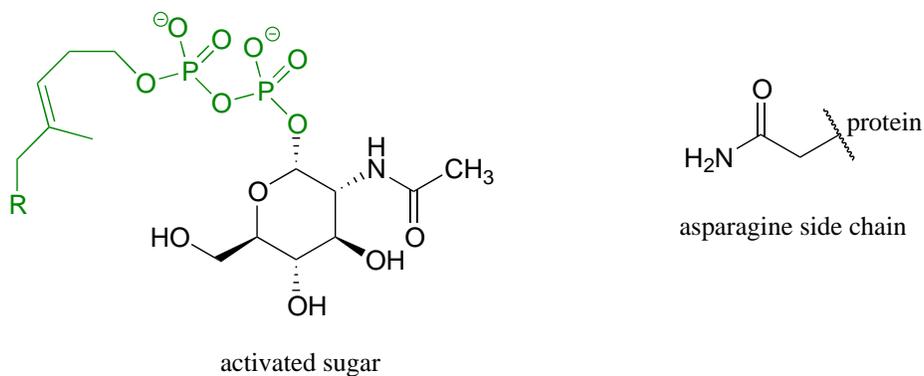
Establishment of the *N*-glycosidic bond in biosynthesis of the pyrimidine ribonucleotides and (U, C and T) also begins with PRPP, but here the ring structure of the nucleotide base part of the biomolecule has already been 'pre-fabricated' in the form of orotate:



Exercise 10.12: We have just seen an illustration of the formation of an *N*-glycosidic bond in a biosynthetic pathway. In the catabolic (degradative) direction, an *N*-glycosidic bond must be broken, in a process which is analogous to the hydrolysis of a glycosidic bond (illustrated earlier). In the catabolism of guanosine nucleoside, the *N*-glycosidic bond is broken by inorganic phosphate (not water!) apparently in a *concerted* (S_N2 -like) displacement reaction (*Biochemistry* 2011, 50, 9158). Predict the products of this reaction, and draw a likely mechanism.



Exercise 10.13: Glycoproteins are proteins that are linked, by glycosidic or *N*-glycosidic bonds, to sugars or carbohydrates through an asparagine, serine, or threonine side chain on the protein. As in other glycosylation and *N*-glycosylation reactions, the hemiacetal of the sugar must be activated prior to glycosidic bond formation. Below is the structure of the *activated* sugar hemiacetal substrate in an asparagine glycosylation reaction

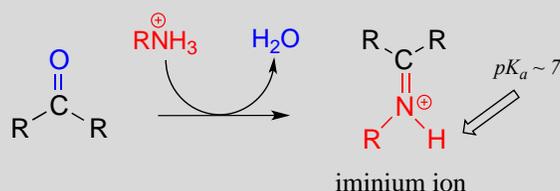


Draw the product of the asparagine glycosylation reaction, assuming inversion of configuration of the anomeric carbon.

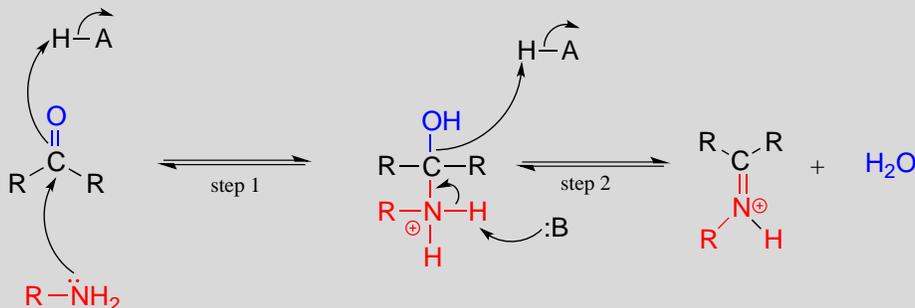
Section 10.5: Imines and iminium ions

The electrophilic carbon atom of aldehydes and ketones can be the target of nucleophilic attack by amines as well as alcohols. The end result of attack by an amine nucleophile is a functional group in which the C=O double bond is replaced by a C=N double bond, and is known as an **imine**. (An equivalent term is '**Schiff base**', but we will use 'imine' throughout this book). Recall from section 7.5B that imines have a pK_a of approximately 7, so at physiological pH they can be accurately drawn as either protonated (**iminium ion** form) or neutral (imine).

Iminium ion formation



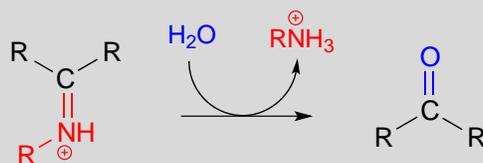
Mechanism (enzymatic):



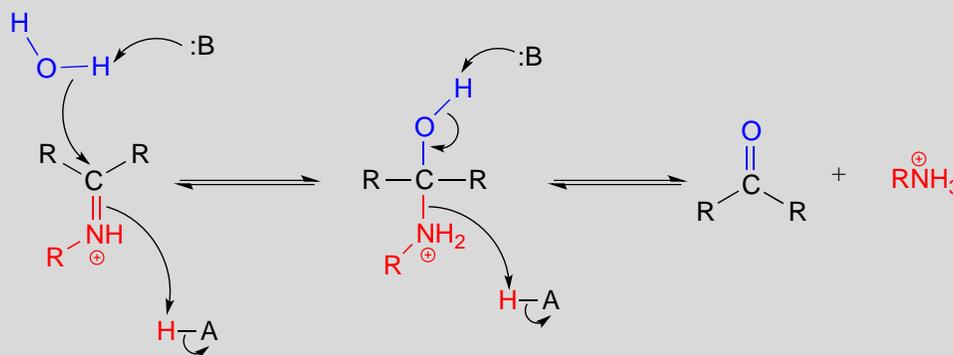
Mechanistically, the formation of an imine involves two steps. First, the amine nitrogen attacks the carbonyl carbon in a nucleophilic addition step (step 1) which is closely analogous to hemiacetal and hemiketal formation. Based on your knowledge of the mechanism of acetal and ketal formation, you might expect that the next step would be attack by a second amine to form a compound with a carbon bound to two amine groups – the nitrogen version of a ketal or acetal. Instead, what happens next (step 2 above) is that the nitrogen lone pair electrons ‘push’ the oxygen off of the carbon, forming a C=N double bond (an iminium) and a displaced water molecule.

The conversion of an iminium back to an aldehyde or ketone is a hydrolytic process (bonds are broken by a water molecule), and mechanistically is simply the reverse of iminium formation:

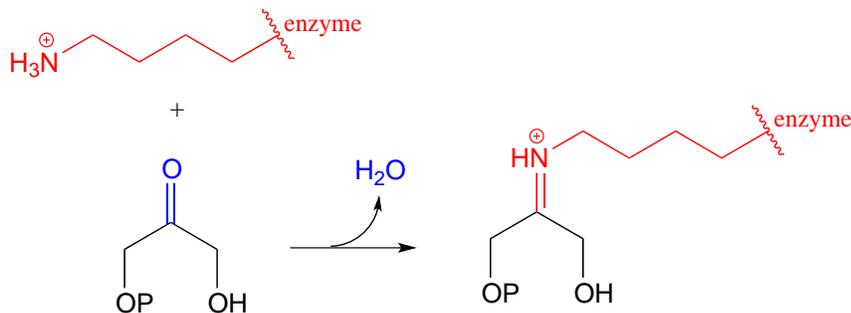
Hydrolysis of an iminium ion:



Mechanism (enzymatic):

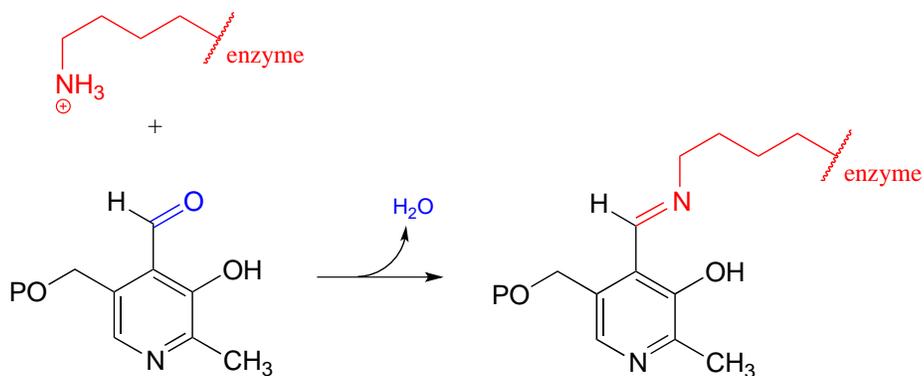


Carbon-carbon bond forming enzymes called aldolases (which we'll cover in detail in chapter 12) often form iminium links between a carbonyl carbon on a substrate and a lysine residue from the active site of the enzyme, as in this aldolase reaction from the Calvin Cycle:

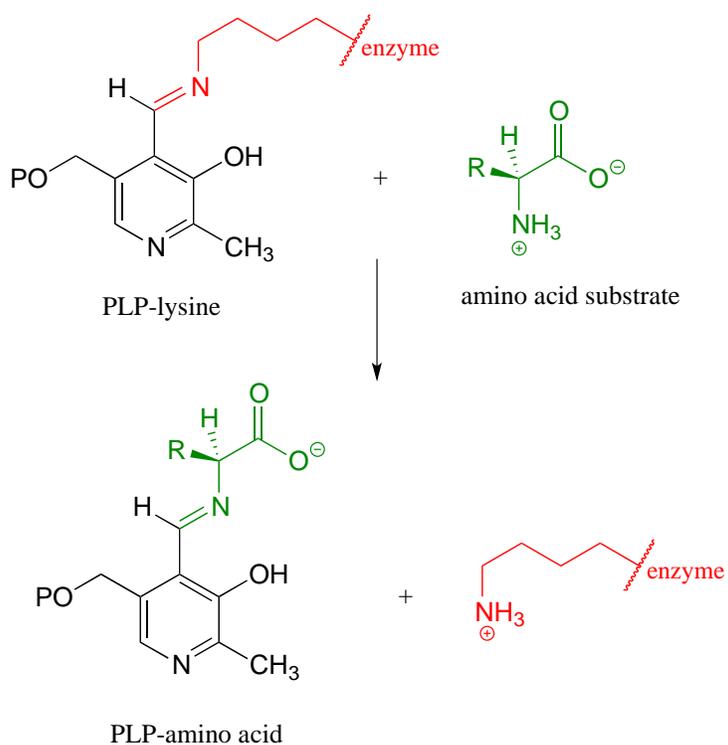


After the carbon-carbon bond forming part of an aldolase reaction is completed, the iminium linkage is hydrolyzed, freeing the product so that it can diffuse out of the active site and allow another catalytic cycle to begin.

In chapter 17, we will learn about reactions that are dependent upon a coenzyme called pyridoxal phosphate (PLP), also known as vitamin B₆. In these reactions, the aldehyde carbon of PLP links to an enzymatic lysine in the active site:



Then, the PLP-lysine imine linkage is traded for an imine linkage between PLP and the amino group on the substrate, in what can be referred to as a **transimination**.

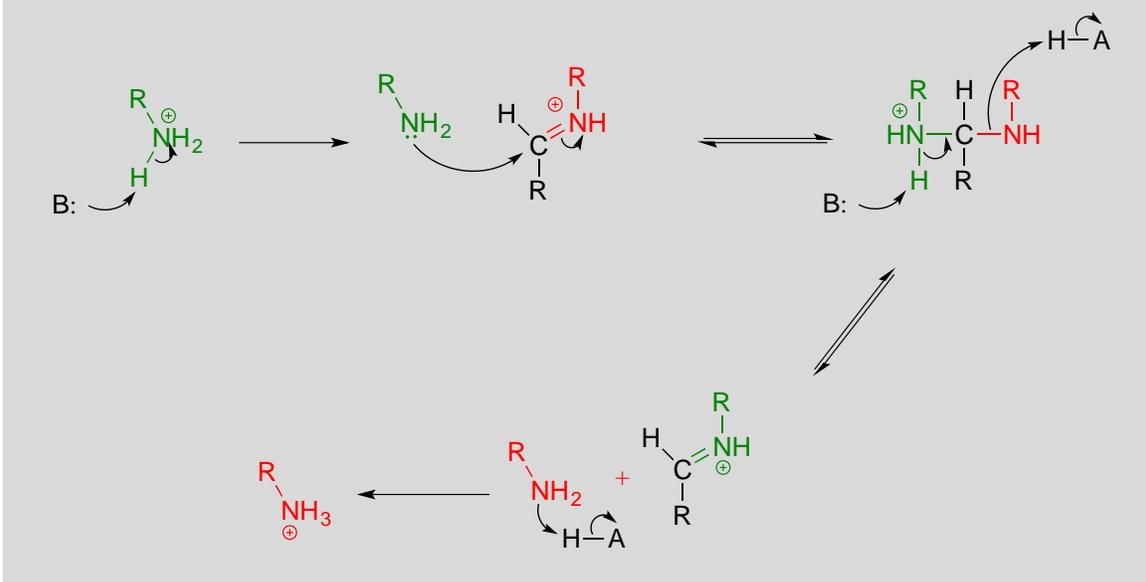


The mechanism for a transimination is very similar to that of imine formation:

Transimination reaction:

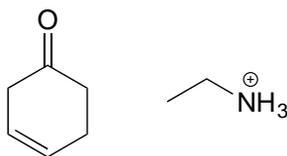


Mechanism:

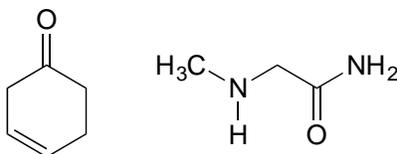


Exercise 10.14: Draw an imine that could be formed between each pair of compounds.

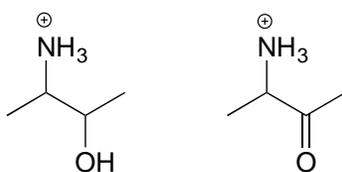
a)



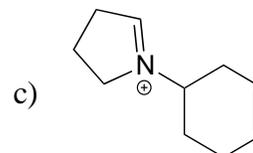
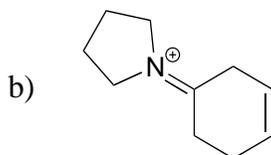
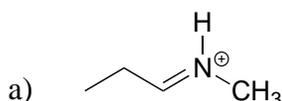
b)



c)

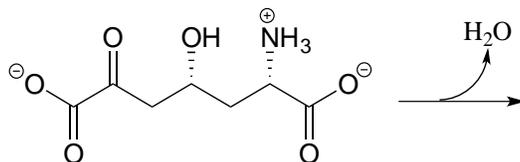


Exercise 10.15: Draw the iminium hydrolysis product for each of the following compounds.

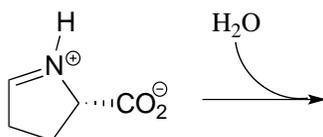


Exercise 10.16:

a) The metabolic intermediate shown below undergoes an intramolecular imine formation as a step in the biosynthesis of lysine (EC 4.3.3.7). Draw the product of this intramolecular imine formation step.

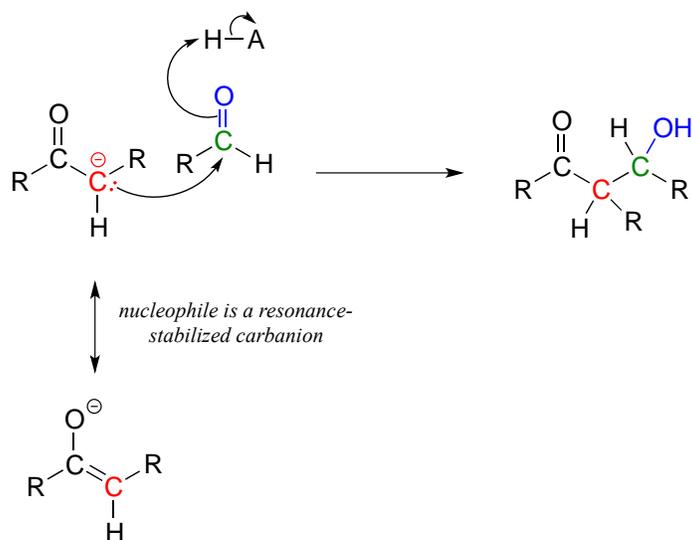


b) Predict the product of this iminium hydrolysis step (EC 2.3.1.117) from the proline degradation pathway.

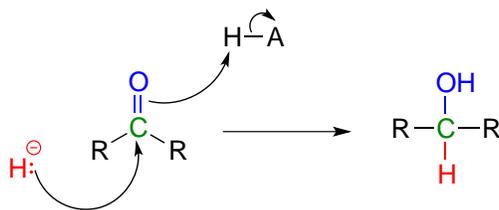


Section 10.6: A look ahead: addition of carbon and hydride nucleophiles to carbonyls

We have seen in this chapter a number of reactions in which oxygen and nitrogen nucleophiles add to carbonyl groups. Other nucleophiles are possible in carbonyl addition mechanisms: in chapters 12 and 13, for example, we will examine in detail some enzyme-catalyzed reactions where the attacking nucleophile is a resonance stabilized carbanion (usually an enolate ion):



Then in chapter 15, we will see how the carbonyl groups on aldehydes and ketones can be converted to alcohols through the nucleophilic addition of what is essentially a hydride (H^-) ion.



Key concepts for review

Before moving on to the next chapter, you should be confident in your ability to:

Recognize aldehyde and ketone groups in organic biomolecules

Draw/explain the bonding picture for aldehyde and ketone groups

Explain why the carbonyl carbon in an aldehyde or ketone is electrophilic

Draw complete curved arrow mechanisms for the following reaction types:

formation of a hemiacetal/hemiketal

collapse of a hemiacetal/hemiketal to revert to an aldehyde/ketone

formation and hydrolysis of an acetal/ketal

formation and hydrolysis of an *N*-glycosidic bond

formation and hydrolysis of an imine

transimination

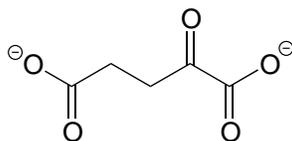
Explain how the carbocation intermediates in glycosidic bond formation and hydrolysis reactions are stabilized by resonance

Explain the stereochemical considerations of a nucleophilic addition to an aldehyde/ketone, especially in the context of glycosidic bond formation. Be able to identify the *re* and *si* faces of an aldehyde, ketone, or imine.

In addition to these fundamental skills, you should develop your confidence in working with end-of-chapter problems involving more challenging, multi-step biochemical reactions.

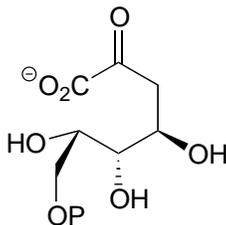
Problems

P10.1 Draw a mechanism showing the formation of an imine linkage between a lysine side chain and α -ketobutyrate (this is the first step in the degradation of lysine, EC 1.5.1.8).

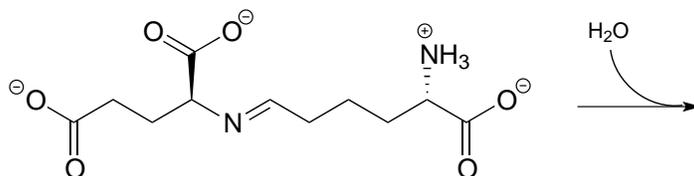


α -ketoglutarate

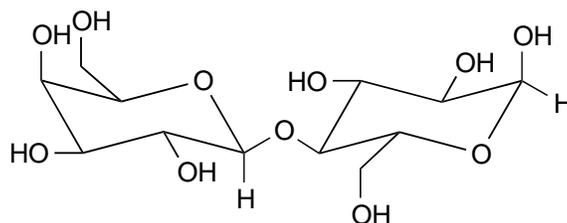
P10.2: Draw four possible cyclic hemiketal isomers of the compound below.



P10.3: A downstream intermediate in the lysine degradation pathway undergoes imine hydrolysis to release two amino acid products (EC 1.5.1.1). Draw a mechanism for this hydrolysis reaction, and show the structures of the two products formed.



P10.4: Below is the structure of lactose, the sugar found in dairy products.

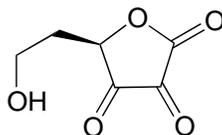


lactose

Lactose is a disaccharide of galactose and glucose. People who are lactose intolerant do not produce enough lactase - the enzyme that hydrolyzes the glycosidic bond linking the two monosaccharides - to be able to fully digest dairy products.

- Draw a likely stabilized carbocation intermediate in the hydrolysis reaction catalyzed by lactase.
- Draw, in the chair conformation, the structure of what you predict would be the most abundant form of the galactose monosaccharide in aqueous solution.
- Is galactose an aldose or a ketose?
- Draw, showing stereochemistry, the open-chain form of galactose.

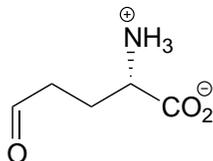
P10.5: You probably know that ascorbic acid (vitamin C) acts as an antioxidant in the body. When vitamin C does its job, it ends up being oxidized to dehydroascorbate, which is usually drawn as shown below, in the so-called tricarbonyl form.



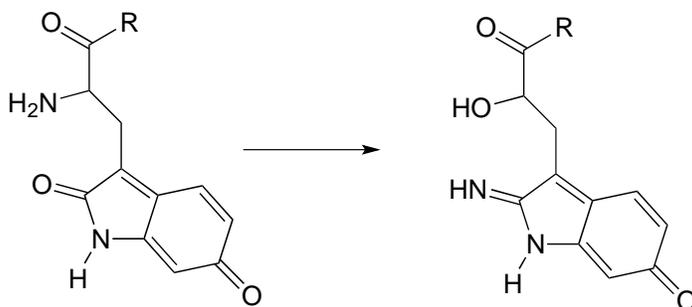
dehydroascorbate
(tricarbonyl form)

Evidence suggests, however, that the most important form of dehydroascorbate in a physiological context is one in which one of the ketone groups is in its hydrated form, and the other is an intramolecular hemiketal (see *Chemical and Engineering News*, Aug. 25, 2008, p. 36). Show the structure of this form of dehydroascorbic acid.

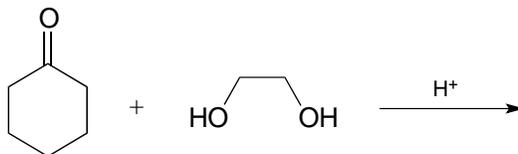
P10.6: The compound below is the product of a ring-opening imine hydrolysis step in the degradation pathway for proline, one of the amino acids. Draw the structure of the starting compound.



P10.7: The rearrangement below was proposed to proceed via imine formation followed by nucleophilic substitution. Propose a mechanism that fits this description.

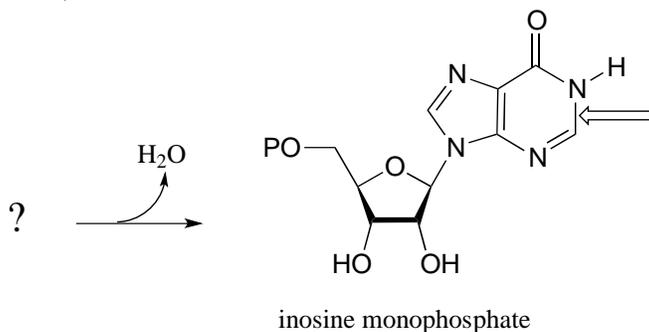


P10.8: The biochemical acetal-forming reactions we learned about in this chapter all require activation of the hemiacetal through phosphorylation. In the organic synthesis lab, non-enzymatic acetal-forming reactions are carried out with a catalytic amount of strong acid, which serves to activate the hemiacetal. Predict the product of the following acetal forming reaction, and propose a reasonable mechanism for the reaction. Remember that the reaction is carried out under acidic conditions, which means that the protonation state of intermediates will be different than biochemical reactions occurring at neutral pH.



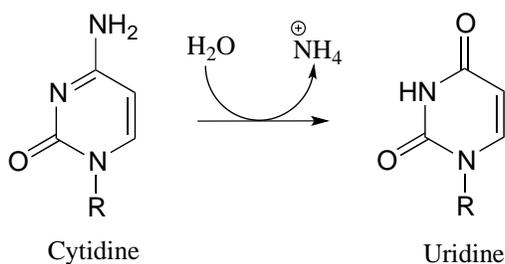
*Problems 9-15 all involve variations on, and combinations of, the nucleophilic addition steps that we studied in this chapter. Although the reactants and/or products may look somewhat different from the simpler aldehydes, acetals, imines, etc. that we used as examples in the chapter, the key steps still involve essentially the same mechanistic patterns. Before attempting these problems, you may want to review **tautomerization** reactions in section 7.6.*

P10.9: The final step in the biosynthesis of inosine monophosphate (IMP, a precursor to both AMP and GMP), is a ring-closing reaction in which a new nitrogen-carbon bond (indicated by an arrow in the structure below) is formed. Predict the starting substrate for this reaction, and propose a mechanism that involves a slight variation on typical imine formation. (EC 3.5.4.10)

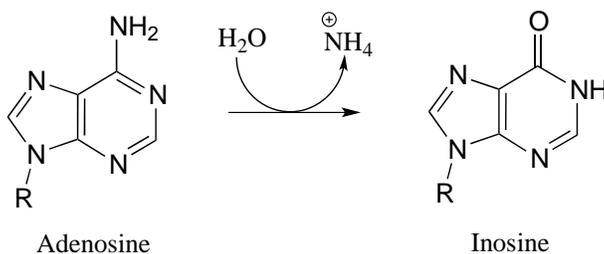


P10.10: Propose a mechanism for these steps in nucleotide metabolism:

a) (EC 3.5.4.5)

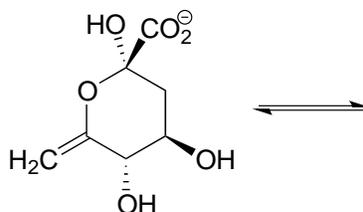


b) (EC 3.5.4.4)

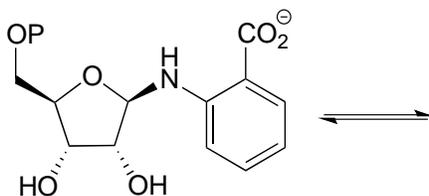


P10.11

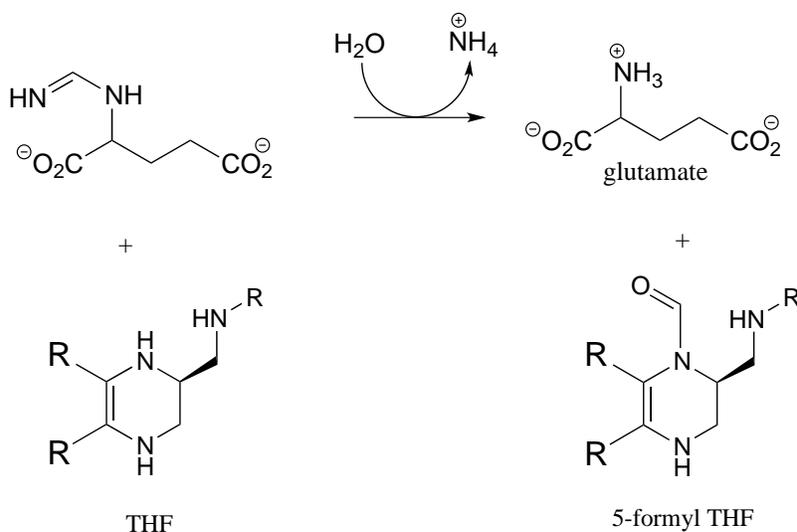
a) Draw the structure (including stereochemistry) of the compound that results when the cyclic hemiketal shown below converts to an open-chain compound with two ketone groups.



b) The compound shown below undergoes a ring-opening reaction to form a species that can be described as both an enol and an enamine. Draw the structure (including stereochemistry) of this product, and a likely mechanism for its formation. (EC 5.3.1.24)

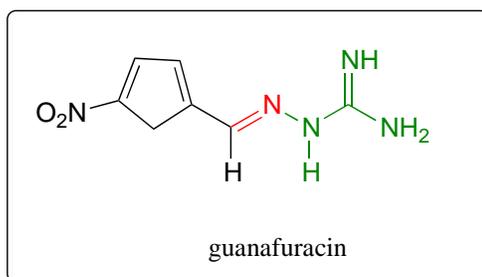
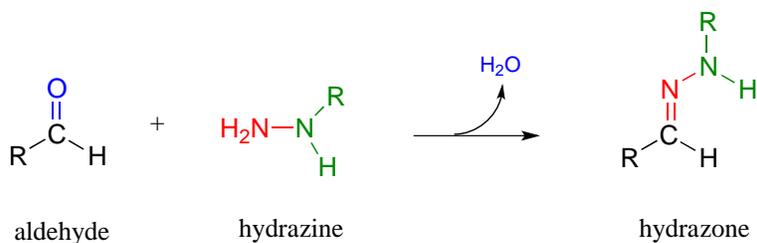


P10.12: Tetrahydrofolate (THF) is a coenzyme that serves as a single-carbon donor in many biochemical reactions. Unlike S-adenosylmethionine (SAM, see section 8.8), the carbon being transferred in a THF-dependent reaction is often part of a carbonyl. Below is a reaction in the histidine degradation pathway (EC 3.5.3.8). The mechanism involved is thought to be an transamination, followed by an imine-to-imine tautomerization, followed by an imine hydrolysis. Propose a reasonable mechanism that fits this description. *Hint:* first identify the carbon atom being transferred.

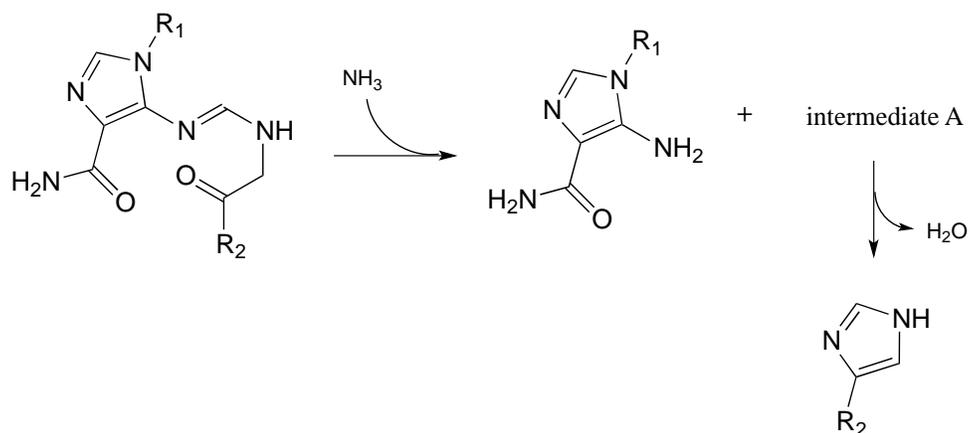


P10.13: Hydrazones are close relatives of imines, formed in reactions between aldehydes/ketones and hydrazines, a functional group containing a nitrogen-nitrogen bond. The mechanism for hydrazone formation is analogous to that of imine formation. Guanafuracin, a known antibiotic compound, is a hydrazone, and can be prepared easily in the laboratory by combining equimolar amounts of the appropriate aldehyde and hydrazine in water (no heat or acid catalyst is required, and the reaction is complete in seconds).

Determine the starting materials required for the synthesis of guanafuracin, and propose a likely mechanism for the reaction.

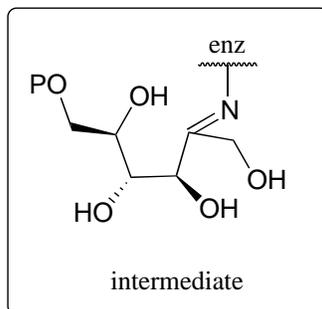
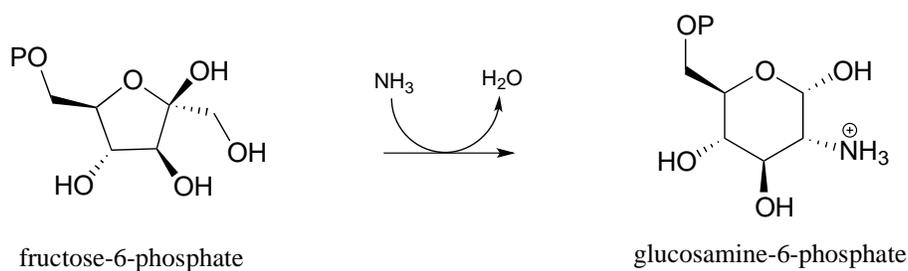


P10.14: Propose reasonable mechanisms for the following steps from the histidine biosynthesis pathway, and predict the structure of intermediate A (which is open-chain, not cyclic).

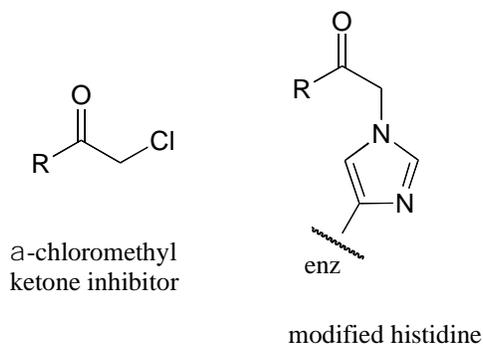


The last several problems are quite challenging!

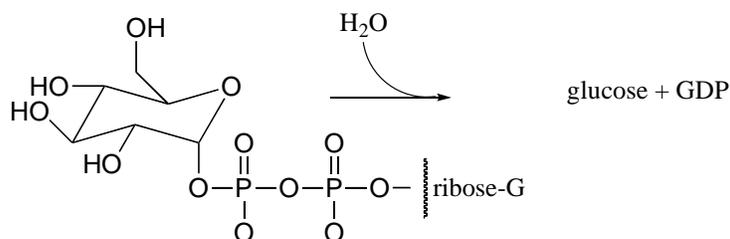
P10.15: Propose a likely mechanism for the synthesis of glucosamine 6-phosphate from fructose-6-phosphate. One of several intermediates is shown. (EC 2.6.1.16.)



P10.16 : α -chloromethyl ketones (structure below) are effective irreversible inhibitors of proteolytic (peptide-bond breaking) enzymes such as chymotrypsin. In these enzymes, a nucleophilic serine plays a key role in the reaction. The mechanism for inactivation of α -chymotrypsin is thought to involve, as a first step, nucleophilic attack by the active site serine on the carbonyl of the inhibitor. However, when the inactivated enzyme is analyzed, an active site histidine rather than the serine, is found to be covalently modified by the inhibitor. The structure of the modified histidine is shown below. The mechanism of inactivation is thought to involve an epoxide intermediate - with this in mind, propose a reasonable mechanism of inactivation.



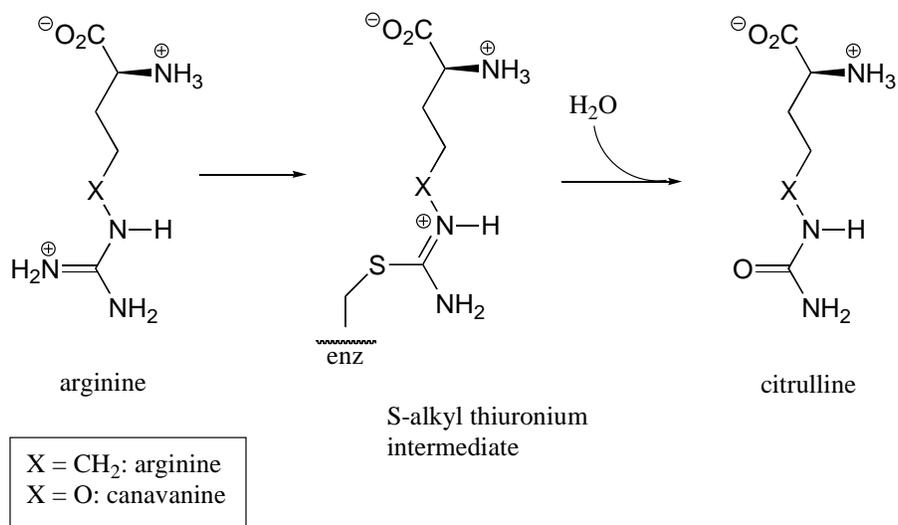
P10.17: An enzyme in *E. coli* bacteria catalyzes the hydrolysis of α -glucose-GDP to glucose.



$^1\text{H-NMR}$ analysis of the reaction in progress showed the initial appearance of a doublet at 4.64 ppm with $J = 7.9$ Hz (the spectrum contained other signals as well, of course). After 20 minutes (at which point the hydrolysis reaction has been complete for some time), another doublet began to appear slightly downfield, this one with $J = 4.0$ Hz. Over time, the strength of the downfield signal gradually increased and that of the upfield signal gradually decreased, until they stabilized at constant levels.

Draw a mechanism for the enzymatic hydrolysis reaction, and correlate your mechanism to the NMR data (including the appearance of the second doublet).

C11.4: Arginine deaminase, an enzyme in the arginine degradation pathway, catalyzes the transformation of (L)-arginine to (L)-citrulline via a covalent substrate-cysteine intermediate.

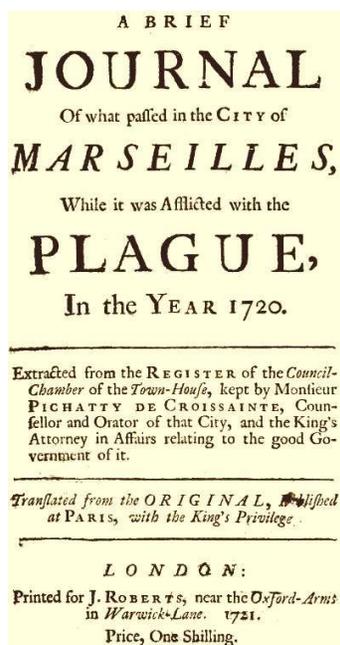


This enzyme is the target for the development of drugs for cancer and immunological diseases such as arthritis. However, rather than completely and permanently shutting down the enzyme (eg. with an irreversible inhibitor), researchers are looking for a way to temporarily 'turn down' the activity of the enzyme. One strategy that has recently been reported involves the use of an oxygen-containing arginine analog, called canavanine, which reacts in the same way as arginine except that the second (hydrolysis) step is very slow. While the enzyme is covalently attached to the inhibitor (in the S-alkyl thiuronium stage), it is inactivated.

- a) Show a mechanism for the reaction catalyzed by arginine deaminase.
- b) Explain how the electronic effect of the oxygen substituent would slow down the hydrolysis step of the reaction, and why the rate of the hydrolysis step is more affected by the oxygen substitution than the S-alkylthiuronium-forming step.

Chapter 11

Nucleophilic acyl substitution reactions



Introduction

The 26th of July, Notice is given to the Sheriffs, that in the Street of *Lescalle*, a Part of the old Town inhabited only by poor People, Fifteen Persons are suddenly fallen sick: They dispatch thither Physicians and Surgeons; they examine into the Distemper, and make Report; some, that 'tis a Malignant Fever; others, a contagious or pestilential Fever, occasioned by bad Food, which Want had long forced those poor Creatures to live upon . . .

The 27th, Eight of those Sick dye; the Sheriffs themselves go to their Houses to cause them to be searched; Buboës [*swelling of the lymph nodes*] are found on Two of them: The Physicians and Surgeons still hold the same Language, and impute the Cause of the Distemper to unwholsome Food. Notwithstanding which, as soon as Night comes, M. *Moustier* repairs to the Place, sends for Servants from the Infirmaries, makes them willingly or by Force, take up the Bodies, with all due Precautions; they are carried to the Infirmaries, where they are buried with Lime; and all the rest of the Night he causes the remaining Sick, and all those of their Houses, to be removed to the Infirmaries.

The 28th, very early in the Morning, Search is made every where for those who had Communication with them, in order to confine them: Other Persons in the same Street fall sick, and some of those who first sicken'd dye. ..

The People who love to deceive themselves, and will have it absolutely not to be the Plague, urge a Hundred false Reasons on that Side. Would the Plague, say they, attack none but such poor People? Would it operate so slowly?

Let them have but a few Days Patience, and they will see all attacked without Distinction, with the swiftest Rage, and the most dreadful Havock, that ever was heard of.

(source: Gutenberg Project
<http://www.gutenberg.org/files/45673/45673-h/45673-h.htm>)

In late May of 1720, a ship arrived in the Mediterranean port city of Marseille, having recently departed from Cyprus and Tripoli. Although several crew members had fallen ill and died during the journey, the ship was allowed to unload after only a very brief quarantine, the result of political pressure on port authorities from local businessmen who wanted quick access to the valuable silk and cotton waiting in the ship's hold.

Along with silk and cotton, the hold carried rats. The rats, in turn, carried fleas. The fleas carried a microscopic mass murderer: *Yersinia pestis*, the species of bacteria that causes bubonic plague.

It is next to impossible to estimate how many people have died from bubonic plague over the course of human history. In the time of the 'Black Death' in the 14th century, it wiped out more than half the population of Europe. In the Great Plague of Marseille in 1720, over 100,000 people succumbed to *Y. pestis* infection in the city and surrounding provinces. At the height of the outbreak, corpses piled up in city streets, and a fortified wall, the '*mur de la peste*' was constructed in an attempt to prevent people from traveling north to the neighboring city of Aix.

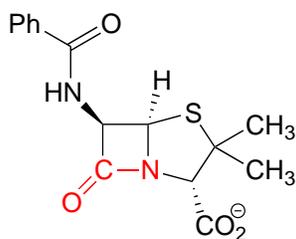
Throughout history, bacteria have been the cause of untold human death and suffering, making the threat posed by more obviously frightening species - lions and bears, spiders and snakes – seem inconsequential by comparison. As recently as the mid-1940s, a minor cut or cold could become a life-threatening event if a bacterial infection were to set in, and even in developed countries, one in twenty infants did not survive to celebrate their first birthday.

Since then, the infant mortality rate in developed countries has declined by a factor of ten. You probably don't worry very much when a small cut on your hand becomes infected. The idea of half of the population of the United States dying in a plague is, in most people's minds, the stuff of zombie movies, not reality. Bacteria are, for now at least, no longer public enemy #1.

How did this happen?

For an answer, we move to a September morning in 1928, in the laboratory of Alexander Fleming, a Scottish bacteriologist working at St. Mary's Hospital in London. As a young man serving in the British Medical Corps during World War I, Fleming saw first-hand how deadly bacteria could be, as he watched countless soldiers in his battlefield hospital die from infected wounds. After returning to civilian life, he began to study *Staphylococci* bacteria, a common source of life-threatening infections in humans, hoping to discover new antibacterial agents that were more effective than those he had used in the war. He spent a lot of his time growing *Staphylococcus* cultures in petri dishes for his experiments, and, notoriously untidy, he tended to leave piles of culture dishes lying around his lab. One morning, he returned from a short vacation to find that one of the cultures he had left out had some mold growing on it. He was about to throw it away, but happened to notice something curious: surrounding the small spot of mold was a circle of clear medium, where no bacteria were growing. He realized that the mold must be secreting something that killed bacteria.

As it turned out, the mold was a of strain called *Penicillium notatum*, and the 'something' killing the bacteria was an organic compound that came to be known as penicillin.



penicillin

Fleming published his findings in the *British Journal of Experimental Pathology*, but made only passing reference to the potential therapeutic value of penicillin. The paper received little attention.

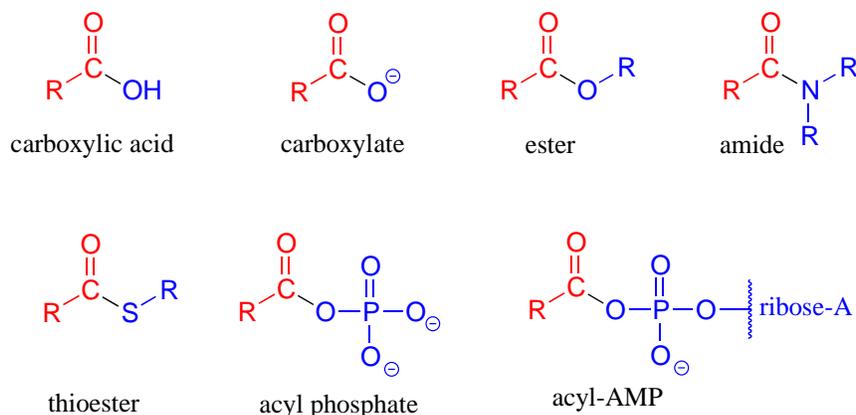
Fast-forward now to early February 1941, with the world once again at war. One morning, a policeman named Albert Alexander living in Oxford, England, had an unfortunate gardening accident. While he was trimming some roses on his day off, his shears slipped and gave him a nasty cut on the side of his mouth. The cut became infected, and after a few days it appeared as if the infection would kill him. Then, he got a visit in his hospital room from some chemists at nearby Oxford University.

For the last few years, the chemists had been hard at work isolating pure penicillin from mold cultures, a tricky job because the compound tends to degrade during purification. It is a feat that Alexander Fleming -who, after all, was a bacteriologist, not a chemist - had never been able to accomplish, but the Oxford researchers had realized how valuable penicillin might be to the war effort, and had finally met with some success. They needed a human subject on whom to test the ability of their compound to treat infected wounds, and Albert was their man for the job. They injected him with penicillin, and within a day his infection cleared up. It was a new day in the history of medicine.

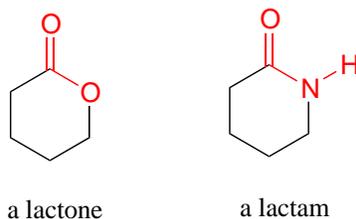
At the heart of a penicillin molecule is an amide functional group - more specifically, a *cyclic* amide, or 'lactam'. To understand how penicillin works at the molecular level as it prevents bacteria from multiplying, we first need to know more about the chemistry of amides and other carboxylic acid derivative functional groups, and a type of organic reaction mechanism called 'nucleophilic acyl substitution'. Understanding the reactivity of carboxylic acid derivative groups will also allow us to appreciate why penicillin is so prone to degradation, and why - very significantly for all of us - the era of not having to worry about bacterial infections may be near an end, as common toxic bacterial species such as *Staphylococcus* develop increasingly robust resistance to antibiotics.

Section 11.1: Carboxylic acid derivatives

The functional groups at the heart of this chapter are called **carboxylic acid derivatives**: they include carboxylic acids themselves, carboxylates (deprotonated carboxylic acids), amides, esters, thioesters, and acyl phosphates.

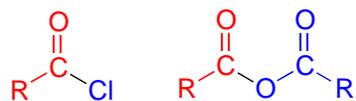


Cyclic esters and amides are referred to as **lactones** and **lactams**, respectively.



Carboxylic acid anhydrides and acid chlorides, which also fall under the carboxylic acid derivative category, are not generally found in biomolecules but are useful intermediates

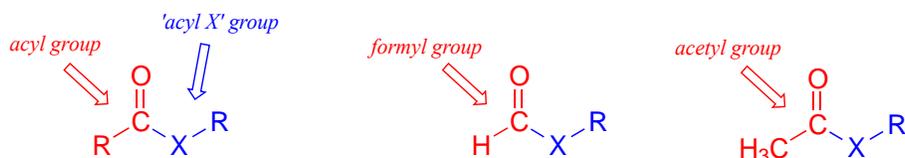
in laboratory synthesis. They are discussed in a section on laboratory reactions at the end of this chapter.



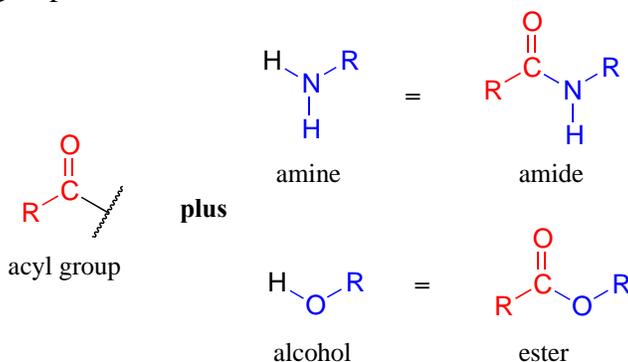
acid chloride

acid anhydride

Carboxylic acid derivatives can be distinguished from aldehydes and ketones by the presence of a group containing an electronegative heteroatom - usually oxygen, nitrogen, or sulfur - bonded directly to the carbonyl carbon. You can think of a carboxylic acid derivative as having two sides. One side is the **acyl** group, which is the carbonyl plus the attached alkyl (R) group. In the specific cases where R is a hydrogen or methyl, chemists use the terms **formyl** and **acetyl** group, respectively. On the other side is the heteroatom-linked group: in this text, we will sometimes refer to this component as the 'acyl X' group (this, however, is *not* a standard term in organic chemistry).



Notice that the acyl X groups are simply deprotonated forms of other functional groups linked to the acyl group: in an amide, for example, the acyl X group is an amine, while in an ester the acyl X group is an alcohol.

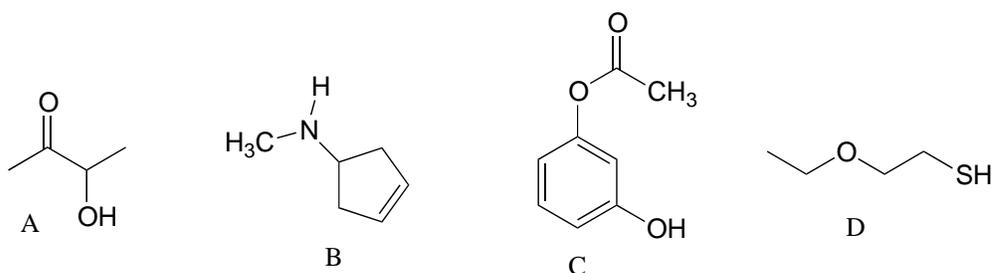


Exercise 11.1: What is the 'acyl X' group in:

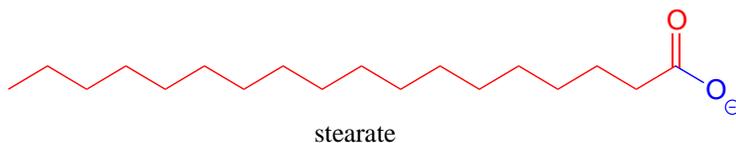
- an acid anhydride?
- a thioester?
- a carboxylic acid?
- an acyl phosphate?

Exercise 11.2: Draw the structures indicated:

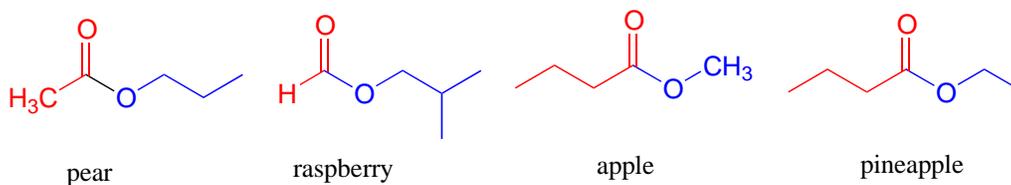
- compound A after it has been acetylated (ie. an acetyl group added)
- compound B after it has been formylated
- compound C after it has been formylated
- Compound D after it has been acetylated



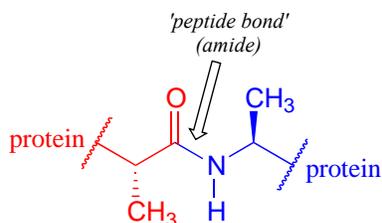
'Fatty acid' molecules such as stearate are carboxylates with long carbon chains for acyl groups.



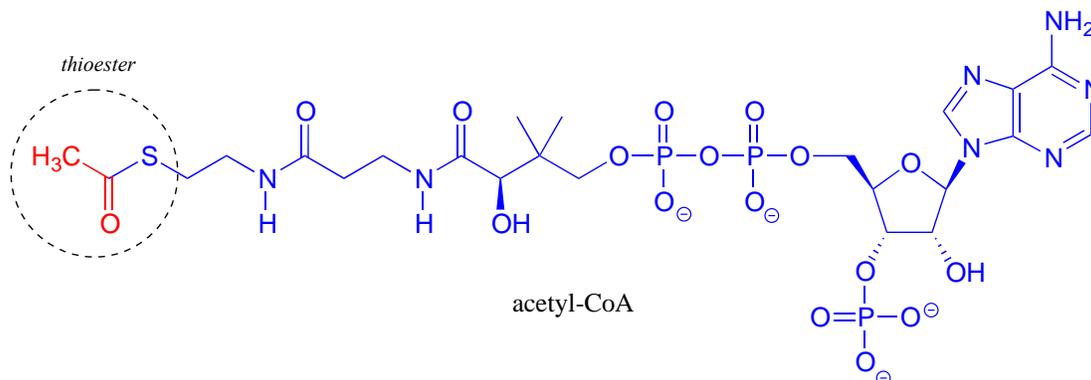
The aromas of many fruits come from small ester-containing molecules:



The 'peptide bonds' that link amino acids together in proteins are amides.

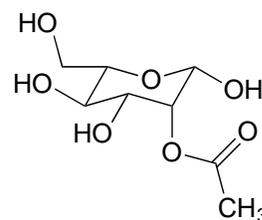
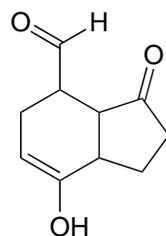
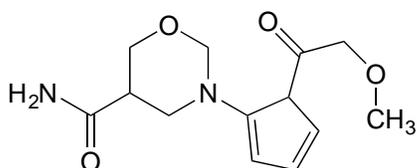
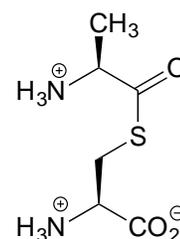
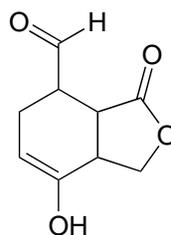
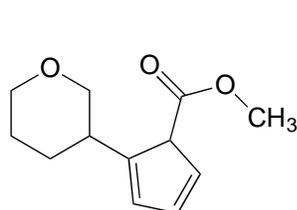


Acetyl-Coenzyme A, a very important two carbon (acetyl group) 'building block' molecule in metabolism, is characterized by reactions at its thioester functional group:



Exercise 11.3: There are two amide groups in acetyl-CoA: identify them.

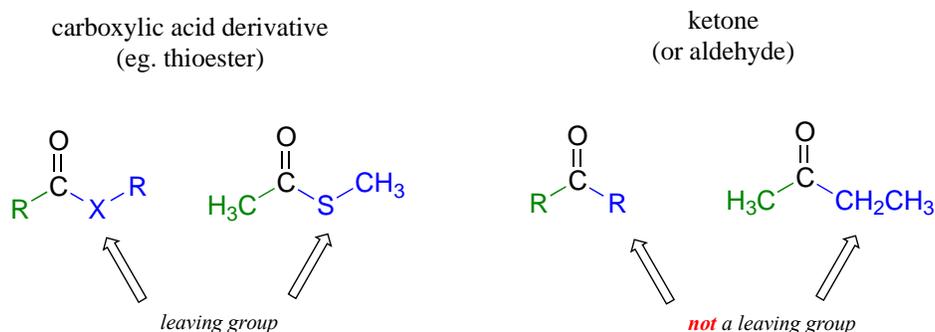
Exercise 11.4: Name all carboxylic acid derivative groups in the molecules below.



Section 11.2: The nucleophilic acyl substitution mechanism

The fact that one of the atoms adjacent to the carbonyl carbon in carboxylic acid derivatives is an electronegative heteroatom – rather than a carbon like in ketones or a

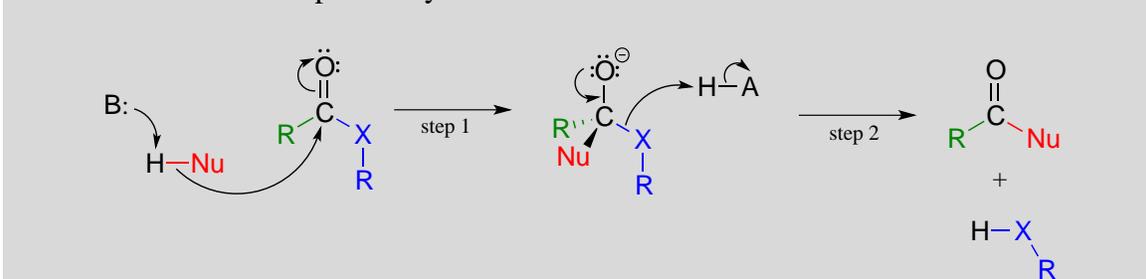
hydrogen like in aldehydes - is critical to understanding the reactivity of carboxylic acid derivatives. The most significant difference between a ketone/aldehyde and a carboxylic acid derivative is that the latter has a potential leaving group - what we are calling the 'acyl X group' - bonded to the carbonyl carbon.



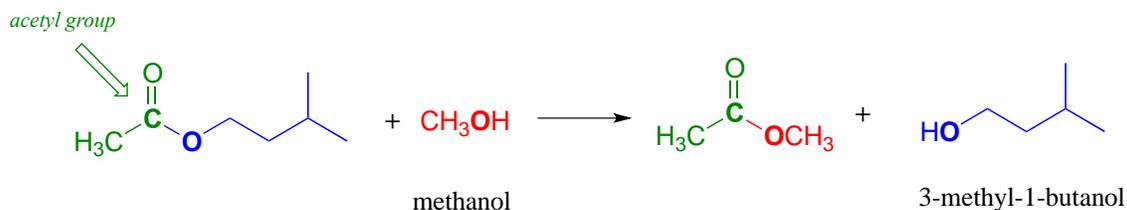
As a result, carboxylic acid derivatives undergo **nucleophilic acyl substitution** reactions, rather than nucleophilic additions like ketones and aldehydes.

A nucleophilic acyl substitution reaction starts with nucleophilic attack at the carbonyl, leading to a tetrahedral intermediate (step 1 below). In step 2, the tetrahedral intermediate collapses and the acyl X group is expelled, usually accepting a proton from an enzymatic acid in the process.

Mechanism for a nucleophilic acyl substitution reaction:

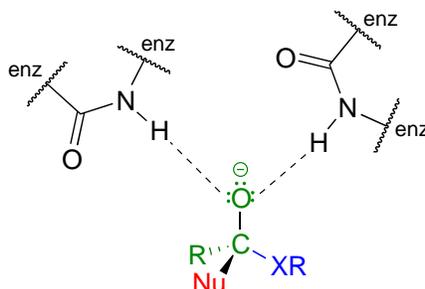


Notice that in the product, the nucleophile becomes the new acyl X group. This is why this reaction type is called a nucleophilic acyl *substitution*: one acyl X group is substituted for another. For example, in the reaction below, one alcohol 'X group' (methanol), substitutes for by another alcohol 'X group' (3-methyl-1-butanol) as one ester is converted to another.



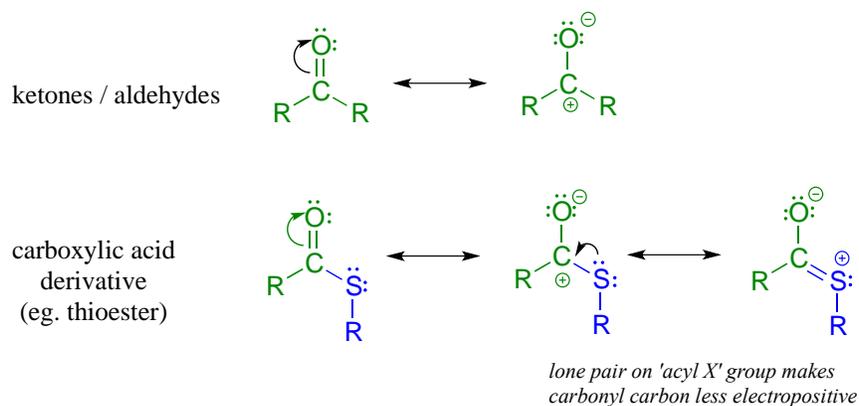
Another way of looking at this reaction is to picture the *acyl* group being transferred from one acyl X group to another: in the example above, the acetyl group (in green) is transferred from 3-methyl-1-butanol (blue) to methanol (red). For this reason, nucleophilic acyl substitutions are also commonly referred to as **acyl transfer** reactions.

Enzymes catalyzing nucleophilic acyl substitution reactions have evolved ways to stabilize the negatively charged, tetrahedral intermediate, thus lowering the activation energy of the first, rate-determining step (nucleophilic attack). The late transition state of the first step resembles the tetrahedral intermediate that results: recall from chapter 6 that the Hammond postulate tells us that anything that stabilizes the tetrahedral intermediate will also stabilize the transition state. In many cases, for example, enzymatic amino acid residues are positioned in the active site so as to provide stabilizing hydrogen bond donating interactions with the negatively-charged oxygen. This arrangement is sometimes referred to in the biochemistry literature as an **oxanion hole**. The figure below shows a tetrahedral intermediate stabilized by hydrogen bond donation from two main chain (amide) nitrogen atoms.



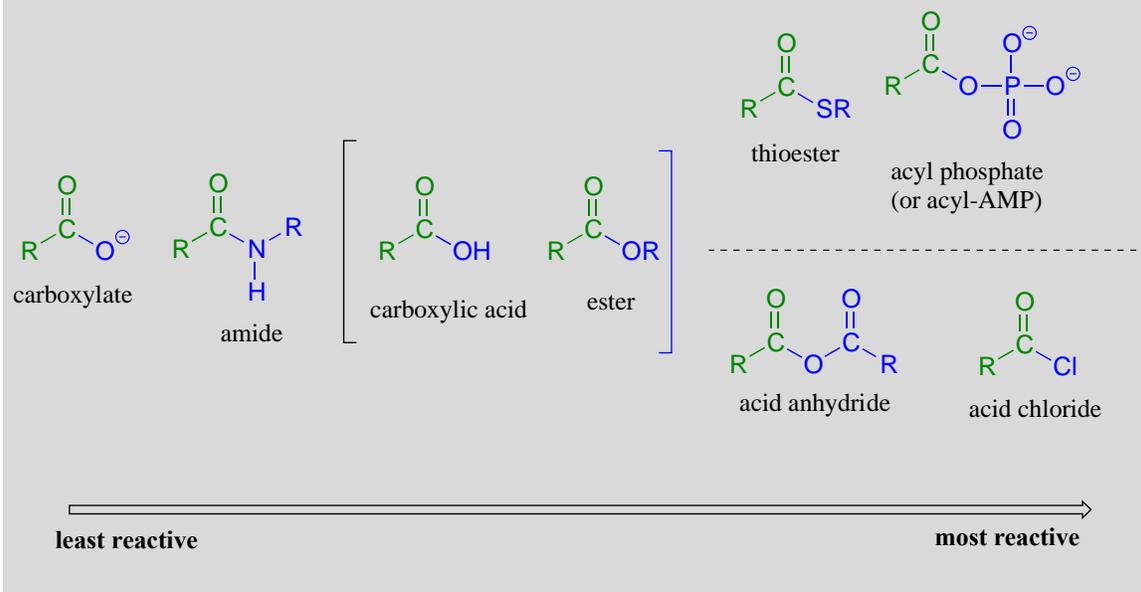
Section 11.3: The relative reactivity of carboxylic acid derivatives

In carboxylic acid derivatives, the partial positive charge on the carbonyl carbon is stabilized by electron donation from nonbonding electrons on the adjacent heteroatom, which has the effect of decreasing electrophilicity.



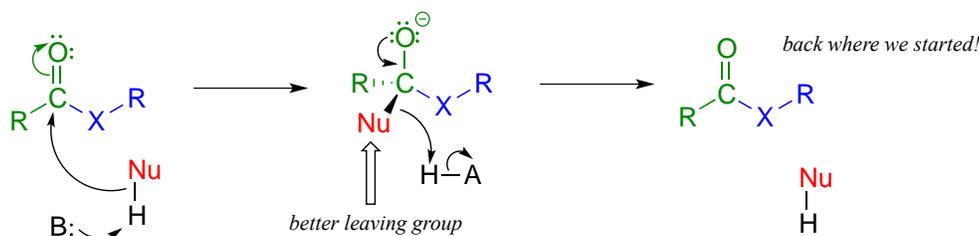
Among the carboxylic acid derivatives, carboxylate groups are the least reactive towards nucleophilic acyl substitution, followed by amides, then carboxylic esters and carboxylic acids, thioesters, and finally acyl phosphates, which are the most reactive among the biologically relevant acyl groups. Acid anhydrides and acid chlorides are laboratory reagents that are analogous to thioesters and acyl phosphates, in the sense that they too are highly reactive carboxylic acid derivatives. Section 11.8 near the end of this chapters includes information about the chemistry of these two reagents.

Relative reactivity of carboxylic acid derivatives:



The reactivity trend of the carboxylic acid derivatives can be understood by evaluating the basicity of the leaving group (acyl X group) - remember from section 8.4 that *weaker bases are better leaving groups*. A thioester is more reactive than an ester, for example, because a thiolate (RS^-) is a weaker base and better leaving group than an alkoxide (RO^-). Recall from chapter 7 that the pK_a of a thiol is about 10, while the pK_a of an alcohol is 15 or higher: a stronger conjugate acid means a weaker conjugate base.

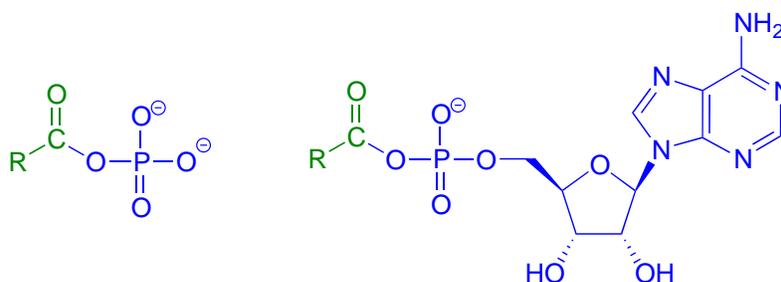
In general, if the incoming nucleophile is a weaker base than the 'acyl X' group that is already there, it will also be the better leaving group, and thus the first nucleophilic step will simply reverse itself and we'll get the starting materials back:



In general, acyl substitution reactions convert higher energy carboxylic acid derivatives into derivatives of lower energy. Thioesters, for example, are often converted directly into carboxylic esters in biochemical reactions, but not the other way around. To go 'uphill' - from a carboxylate to a thioester, for example, requires the 'coupling' of the uphill reaction to an energetically favorable reaction. We will see how this works in the next section.

Section 11.4: Acyl phosphates

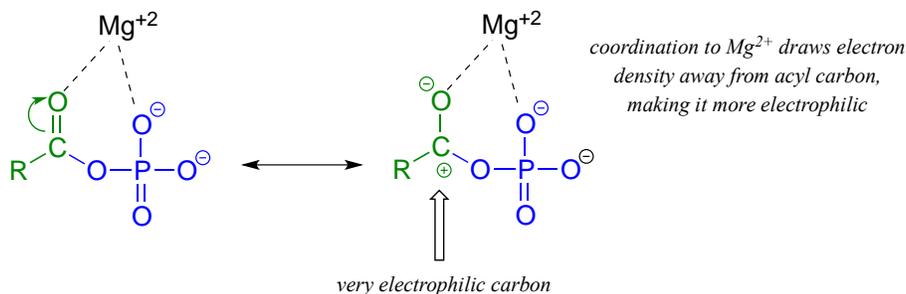
Acyl phosphates, because they are so reactive towards acyl substitutions, are generally seen as reaction intermediates rather than stable metabolites in biochemical pathways. Acyl phosphates usually take one of two forms: a simple acyl monophosphate, or acyl-adenosine monophosphate.



acyl monophosphate

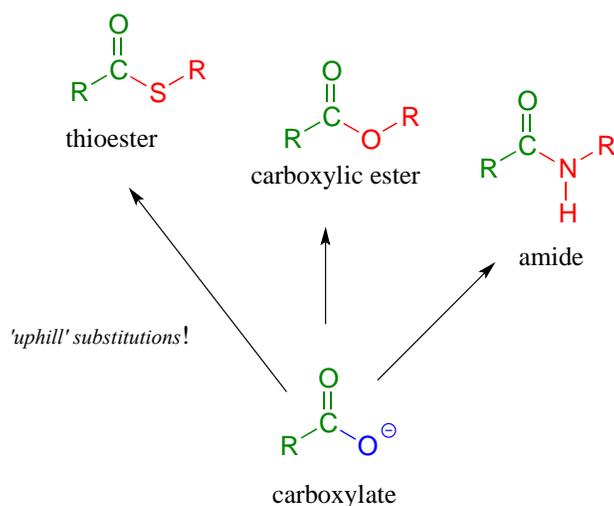
acyl adenosine monophosphate
(acyl AMP)

Both forms are highly reactive to acyl substitution reactions, and are often referred to as '**activated acyl groups**' or '**activated carboxylic acids**' for reasons that will become clear soon. The tendency of phosphates to form stabilizing complexes with one or more magnesium ions in an enzyme's active site contributes in a large way to the reactivity of acyl phosphates.



A magnesium ion acts as a Lewis acid, accepting electron density from the oxygen end of the acyl carbonyl bond, which greatly increases the degree of partial positive charge - and thus the electrophilicity - of the carbonyl carbon. The magnesium ion also balances negative charge on the phosphate, making it a weak base and excellent leaving group.

We have already learned that the carboxylate functional group is the *least* reactive substrate for an enzyme-catalyzed acyl substitution reactions. In biology, though, carboxylates are frequently transformed into thioesters, carboxylic esters, and amides, all of which are higher in energy, meaning that these transformations are thermodynamically 'uphill'.

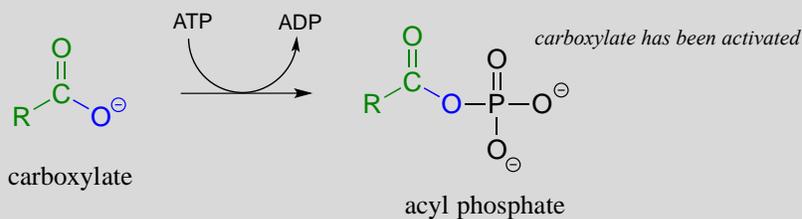


How are these uphill substitutions accomplished? They are not carried out directly: like all thermodynamically unfavorable reactions in biochemistry, they are linked to an energy-releasing, 'downhill' reaction. In this case, (and many others), the linked reaction that 'pays for' the uphill reaction is hydrolysis of ATP.

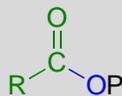
In order to undergo an acyl substitution reaction, a carboxylate must first be activated by phosphorylation. You are already familiar with this phosphoryl group transfer process from chapter 9.

In many cases, enzymes activate a carboxylate group by converting it to an acyl phosphate (the most reactive of the carboxylic acid derivatives), at the expense of an ATP: the mechanism for this type of transformation is shown in Section 9.5.

Formation of an acyl phosphate (see section 9.5 for the complete mechanism):

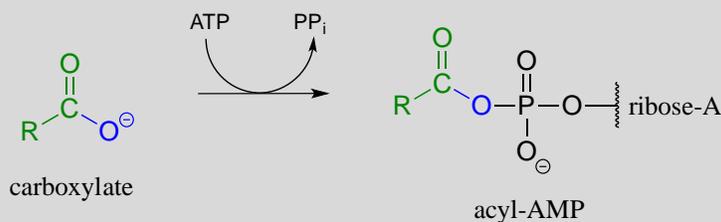


abbreviation:

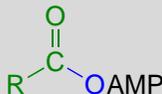


As a common alternative, some enzymatic reactions begin with the conversion of a carboxylate to an acyl-AMP intermediate:

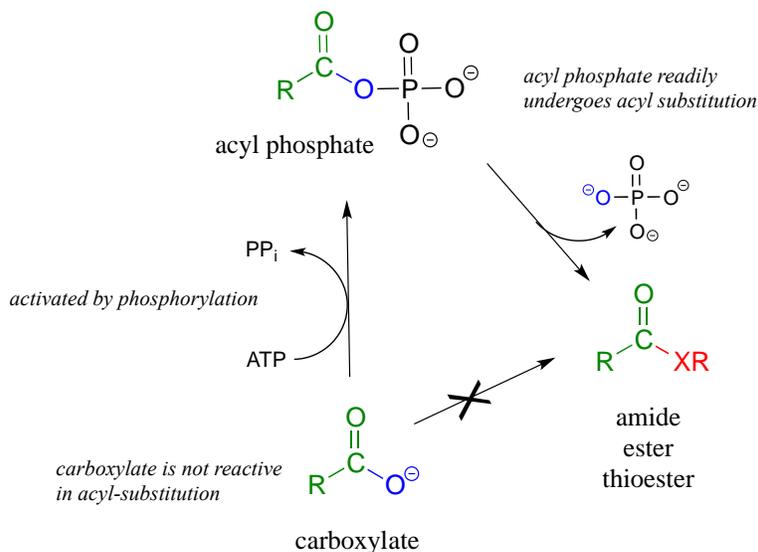
Formation of an acyl -AMP (see section 9.5 for the complete mechanism):



abbreviation:



In either case, once the carboxylate group has been activated, the reactive acyl phosphate/acyl-AMP intermediate can go on to act as the electrophile in an energetically favorable nucleophilic acyl substitution reaction.



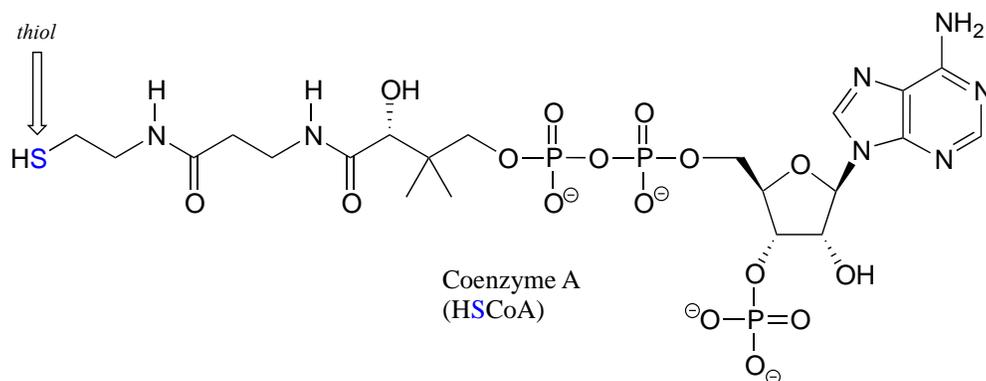
You have probably heard ATP referred to as the 'energy currency' molecule. The reactions in this section provide a more concrete illustration of that concept. A lower-energy group (a carboxylate) is converted to a higher-energy group (a thioester, for example) by 'spending' a high-energy ATP.

Section 11.5: Formation of thioesters, carboxylic esters, and amides

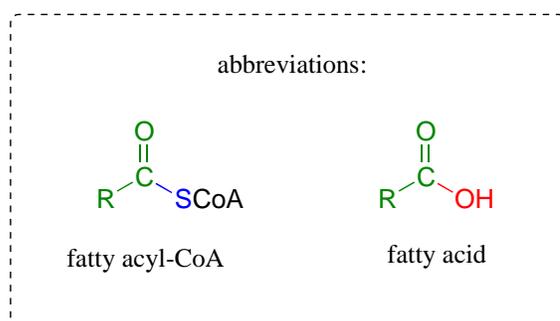
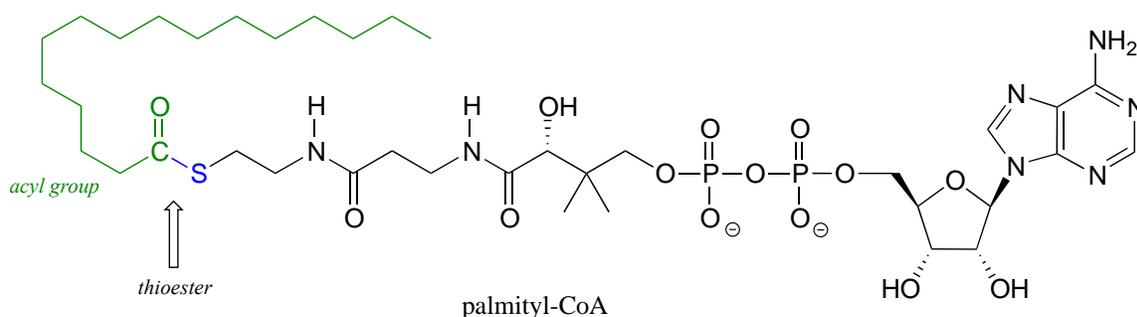
11.5A: Thioester formation

Thioesters, which are themselves quite reactive in acyl substitution reactions (but less so than acyl phosphates), play a crucial role in the metabolism of fatty acids. The 'acyl X group' in a thioester is a thiol.

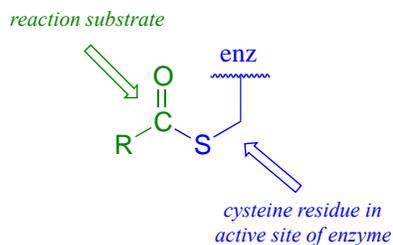
Coenzyme A is a thiol-containing coenzyme that plays a key role in metabolism. Coenzyme A is often abbreviated 'HSCoA' in order to emphasize the importance of the thiol functionality.



Coenzyme A serves as a 'carrier' group in lipid biosynthesis, and is attached by a thioester linkage to growing fatty acid chains. Palmityl is shown below as an example of a typical fatty acyl-CoA thioester.

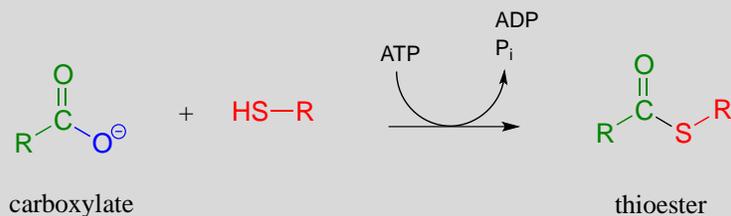


As we look at reactions involving thioesters in this and future sections, we will frequently see Coenzyme A playing a key role. We will also see the formation and breaking of thioester linkages between an acyl group and other thiol-containing species, such as a cysteine residue on the enzyme:



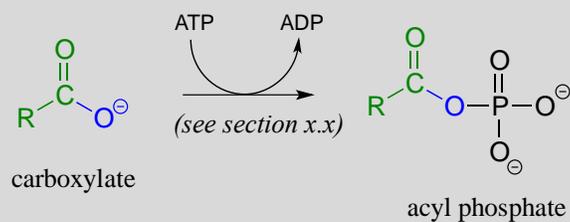
The term 'thioesterification' refers to the formation of a thioester functional group. In a typical biochemical thioesterification reaction, a carboxylate is first converted into an acyl phosphate (in other words, it is activated), then the acyl phosphate undergoes an acyl substitution reaction with a thiol nucleophile.

Thioesterification reaction:

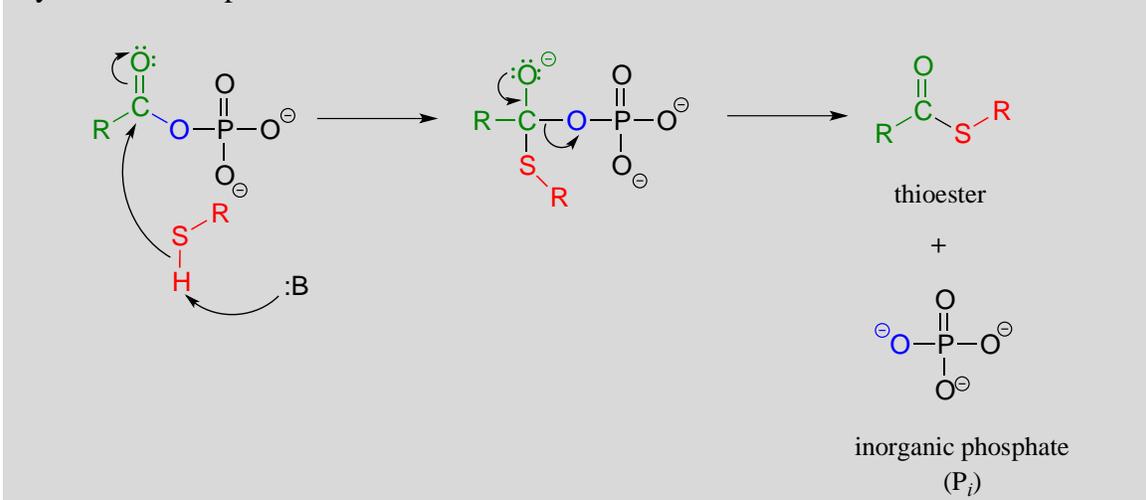


Mechanism:

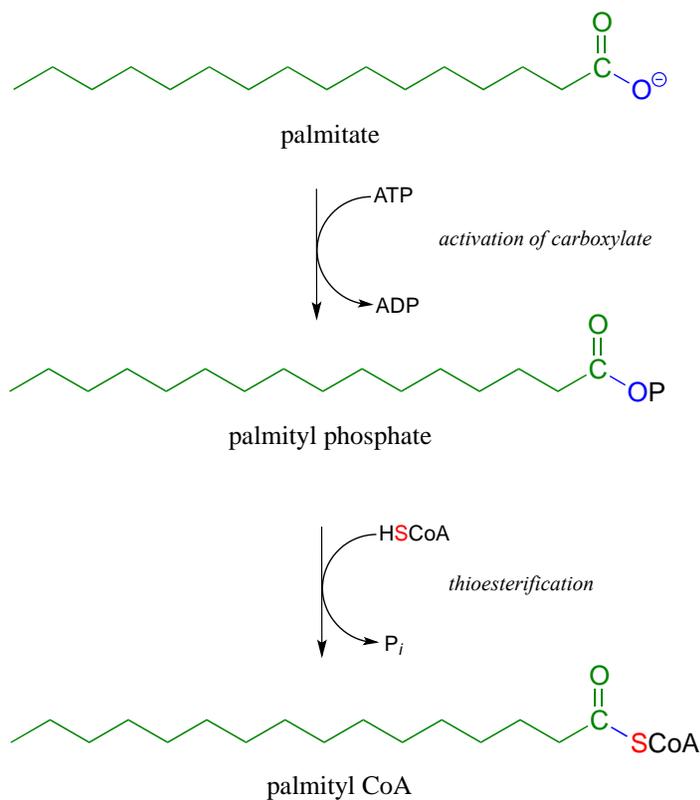
activation phase:



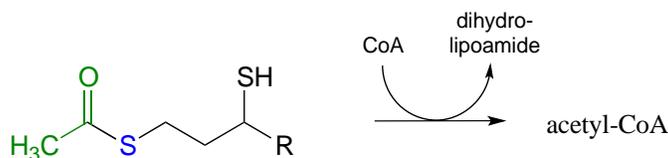
acyl substitution phase:



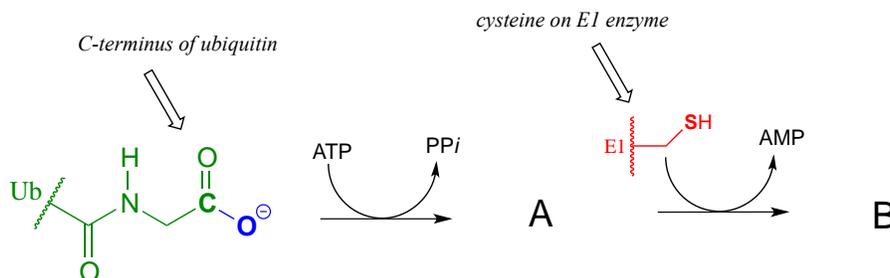
Fatty acids such as palmitate, from fats and oils in your food, are converted to a coenzyme A thioester prior to being broken down by the fatty acid degradation pathway.



Exercise 11.5: The pyruvate dehydrogenase complex (EC 1.2.4.1) catalyzes one of the most central of all central metabolism reactions, the conversion of pyruvate to acetyl-CoA, which links the glycolytic pathway to the citric acid (Krebs) cycle. The reaction is quite complex, and we are not yet equipped to follow it through from start to finish (we will finally be ready to do this in section 17.3). The final step, however, we can understand: it is a transthioesterification, involving a dithiol coenzyme called dihydrolipoamide and coenzyme A. Given the information below, draw out a reasonable mechanism for the reaction.



Exercise 11.6: Ubiquitin is a protein which plays a key role in many cellular processes by reversibly attaching to other proteins, thus altering or regulating their function. Recently, a team of researchers uncovered details of the mechanism by which ubiquitin (abbreviated Ub) is transferred by the ubiquitin activating enzyme (abbreviated E1) to target proteins. In the first part of this process, the carboxy terminus of ubiquitin is linked to a cysteine side chain on E1, as shown in the incomplete reaction sequence below. Complete the figure by drawing the structures of species A and B.



11.5B: Formation of esters

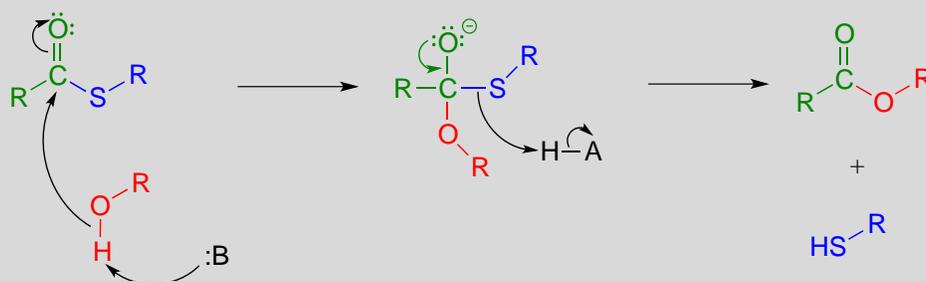
Esterification refers to the formation of a new ester functional group.

In a typical biochemical esterification, a thioester is subjected to nucleophilic attack from an alcohol, leading to the formation of an ester and a thiol.

Esterification reaction (from thioester):

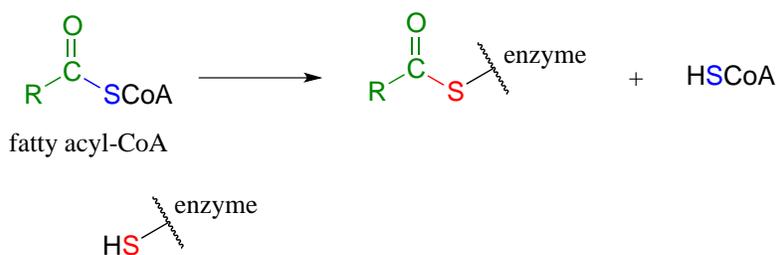


Mechanism:

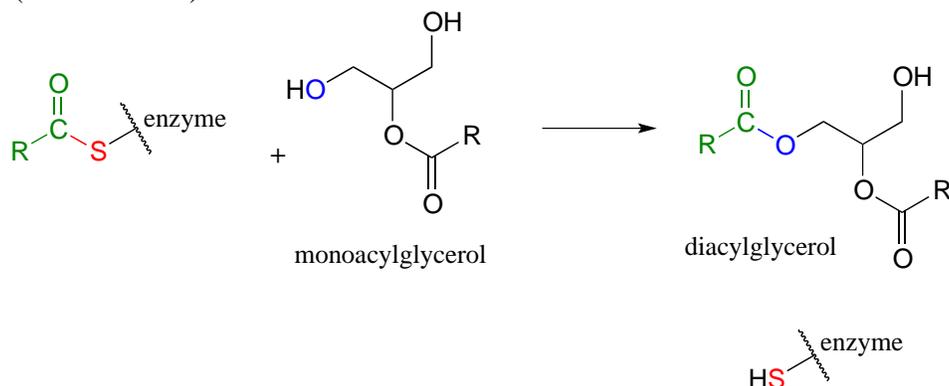


The reaction below is from the synthesis of triacylglycerol, the form in which fat is stored in our bodies.

Phase 1 (transthioesterification):



Phase 2 (esterification):

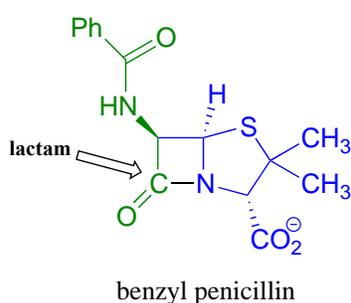


The reaction, catalyzed by monoacylglycerol acyltransferase (EC 2.3.1.22), begins (phase 1 above) with a preliminary transthioesterification step in which the fatty acyl group is transferred from coenzyme A to a cysteine residue in the active site of the enzyme. Recall

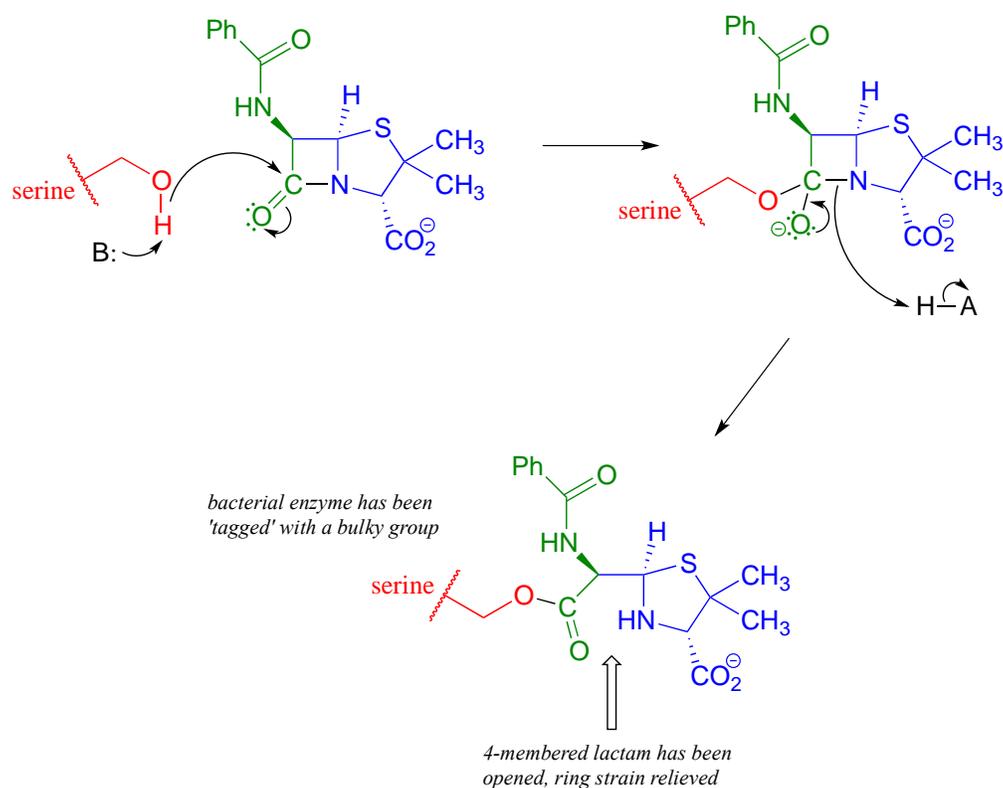
that it is a common strategy for enzymes to first form a covalent link to one substrate before catalyzing the 'main' chemical reaction.

In phase 2 of the reaction, the fatty acyl group is now ready to be transferred to glycerol, trading its thioester linkage to the cysteine for a new ester linkage to one of the alcohol groups on glycerol.

An esterification reaction has tremendous importance in the history of drug development, a story that we heard in the introduction to this chapter. The discovery of **penicillin** was arguably one of the most important events in the history of modern medicine. The key functional group in penicillin is the four-membered lactam (recall that a lactam is a cyclic amide).



Penicillin, and later generations of antibiotic drugs, have saved countless lives from once-deadly bacterial infections. The elucidation of the chemical mechanism of penicillin action was also a milestone in our developing understanding of how drugs function on a molecular level. We now know that penicillin, and closely related drugs such as ampicillin and amoxicillin, work by inhibiting an enzyme that is involved in the construction of the peptide component bacterial cell walls. The details of the wall-building reaction itself are outside the scope of this discussion, but it is enough to know that the process involves the participation of a nucleophilic serine residue in the active site of the enzyme. The penicillin molecule is able to enter the active site, and once inside, the lactam group serves as an electrophilic 'bait' for the nucleophilic serine:



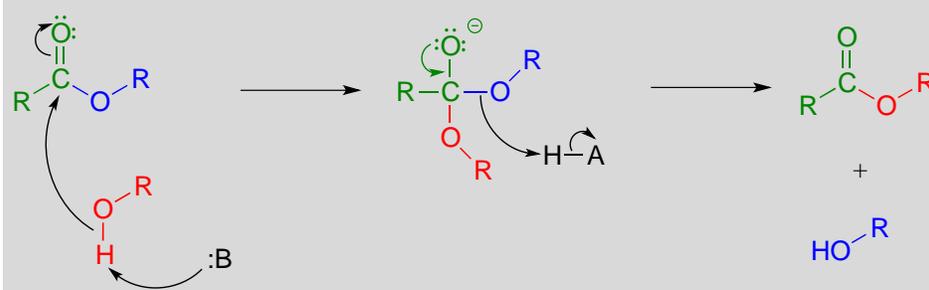
Although you might expect that an amide-to-ester conversion such as what is shown above would be energetically unfavorable based on the reactivity trends we have learned, this lactam is in fact much more reactive than an ordinary amide group due to the effect of ring strain: recall from section 3.2 that four-membered rings are highly strained, and considerable energy is released when they are opened.

Ring strain also accounts for why penicillin has a tendency to degrade: when in contact with water, the lactam will spontaneously hydrolyze over time, which opens the ring and forms a carboxylate group.

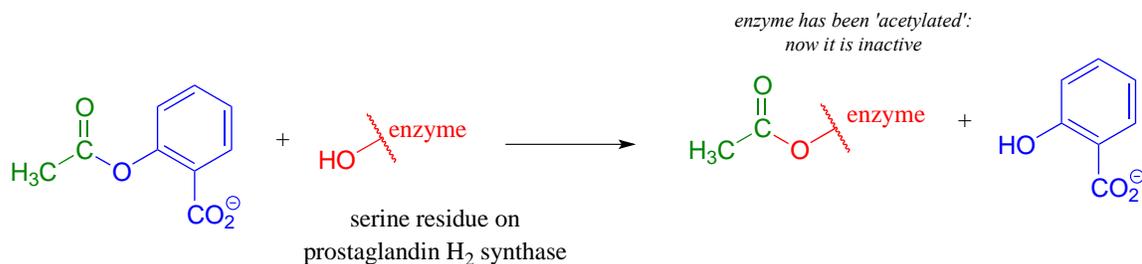
Unfortunately, many strains of bacteria have acquired an enzyme called **β -lactamase** (EC 3.5.2.6), that catalyzes rapid hydrolysis of the lactam ring in penicillin-based drugs, rendering them inactive. These bacteria are consequently resistant to penicillin and related antibiotics. As you are probably aware, the evolution of drug resistance in bacteria is a major, world-wide health problem, and scientists are engaged in a constant battle to develop new antibiotics as the older ones become less and less effective.

In a **transesterification** reaction, one ester is converted into another by an acyl substitution reaction.

Mechanism for a transesterification reaction:



If studying organic chemistry sometimes gives you a headache, you might want to turn to a transesterification reaction for help. Prostaglandins are a family of molecules that promote a wide range of biological processes, including inflammation. Acetylsalicylic acid, commonly known as aspirin, acts by transferring - through a transesterification reaction - an acetyl group to a serine residue on the enzyme responsible for the biosynthesis of prostaglandin H₂ (one member of the prostaglandin family).



Acetylation of this serine blocks a channel leading to the active site, effectively shutting down the enzyme, impeding prostaglandin production, and inhibiting the inflammation process that causes headaches.

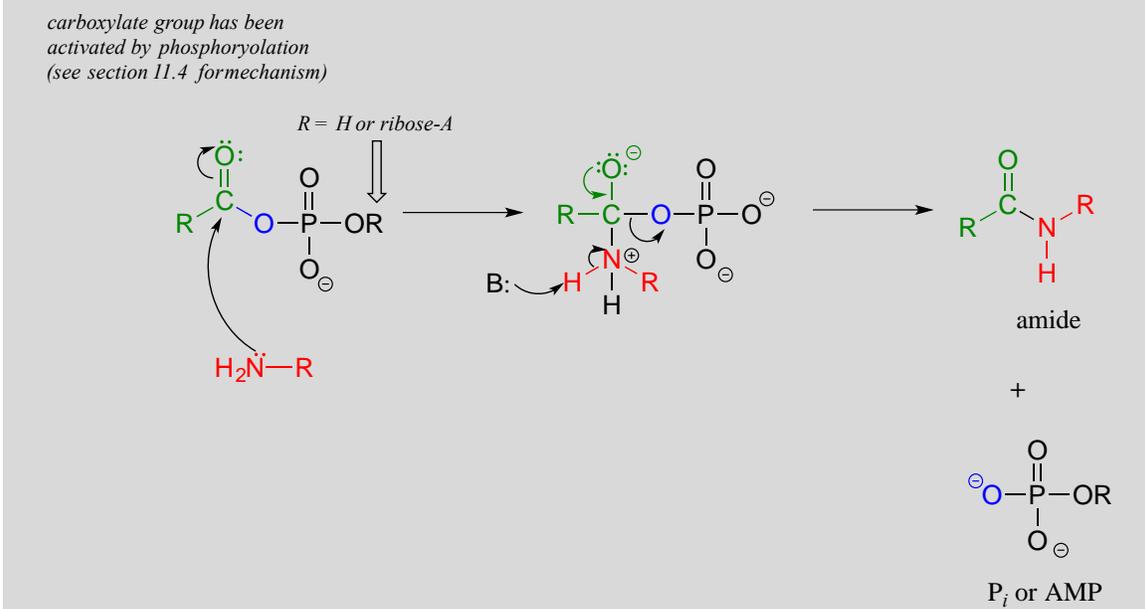
In section 11.8, we will see two laboratory acyl substitution reactions that lead to the formation of aspirin and ibuprofen.

Exercise 11.7: Discuss the key structural feature of aspirin that makes it so effective at transferring its acetyl group - in other words, why is the ester group in aspirin more reactive than a typical ester?

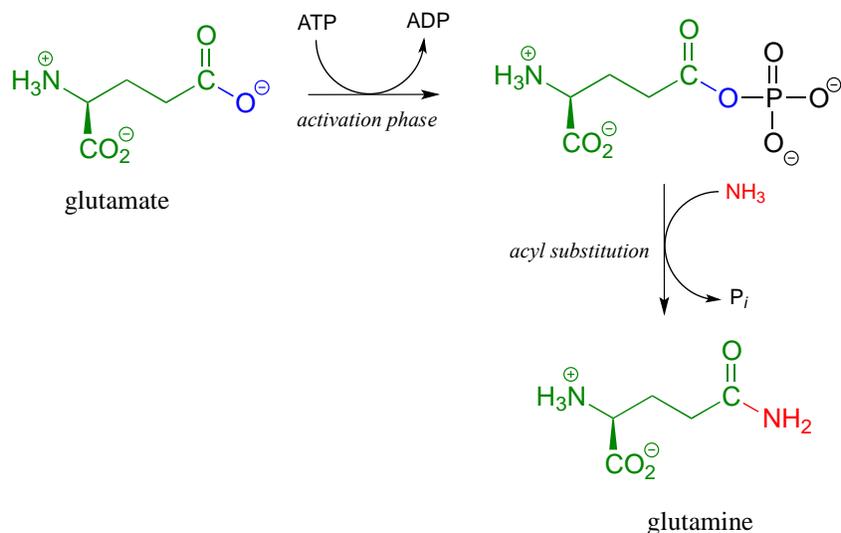
11.5C: Amide formation

An activated carboxylate group (in other words, acyl phosphate or acyl-AMP) can be converted to an amide through nucleophilic attack by an amine.

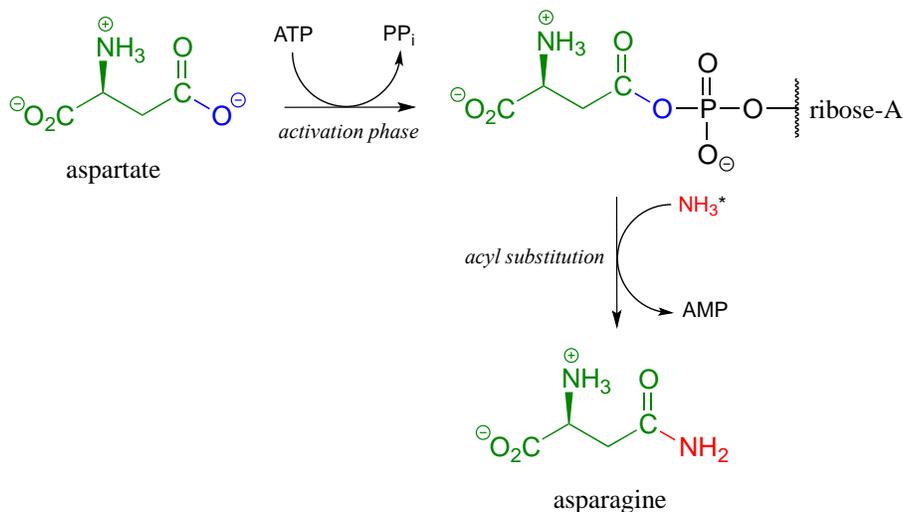
Mechanism for amide formation:



The amino acid biosynthesis pathways provide examples of amide formation in biology. The amino acid glutamine is synthesized in most species by converting the carboxylate side chain of glutamate (another amino acid) to an amide, after first activating the carboxylate by monophosphorylation: (EC 6.3.1.2)

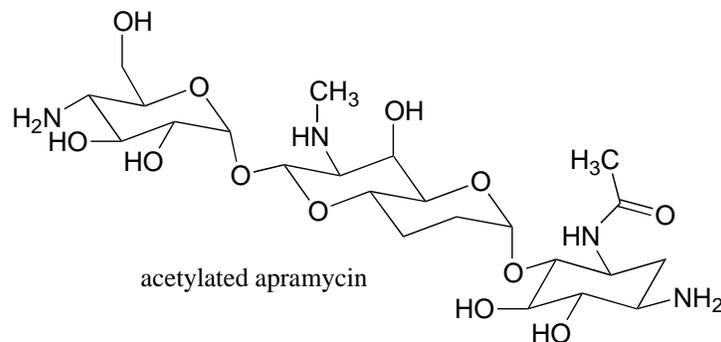


A similar process takes place in the synthesis of asparagine from aspartate, except that the activated carboxylate in this case is an acyl-AMP:



*In the asparagine synthesis reaction, the ammonia nucleophile actually comes from hydrolysis of a glutamine molecule.

Exercise 11.8: An enzyme in bacteria is thought to be responsible for resistance to a class of antibiotics that includes apramycin, ribostamycin and paromomycin. The enzyme catalyzes acetylation of the antibiotic compound with acetyl-CoA as an additional substrate. The structure of acetylated apramycin is shown below.

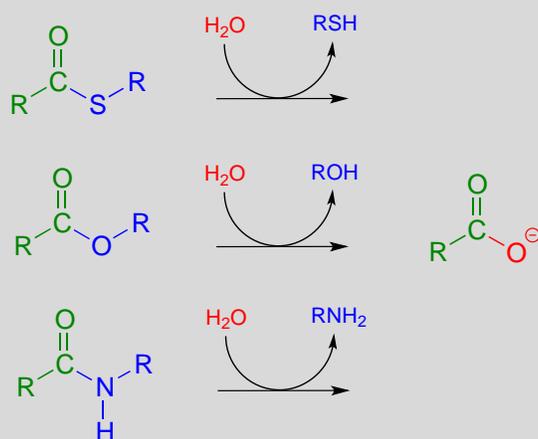


- Identify the acetyl group that has been transferred to apramycin, (and thus inactivating it).
- What functional group acts as an acetyl group *donor*? What functional group acts as an acetyl group?
- What is the coproduct of the reaction?

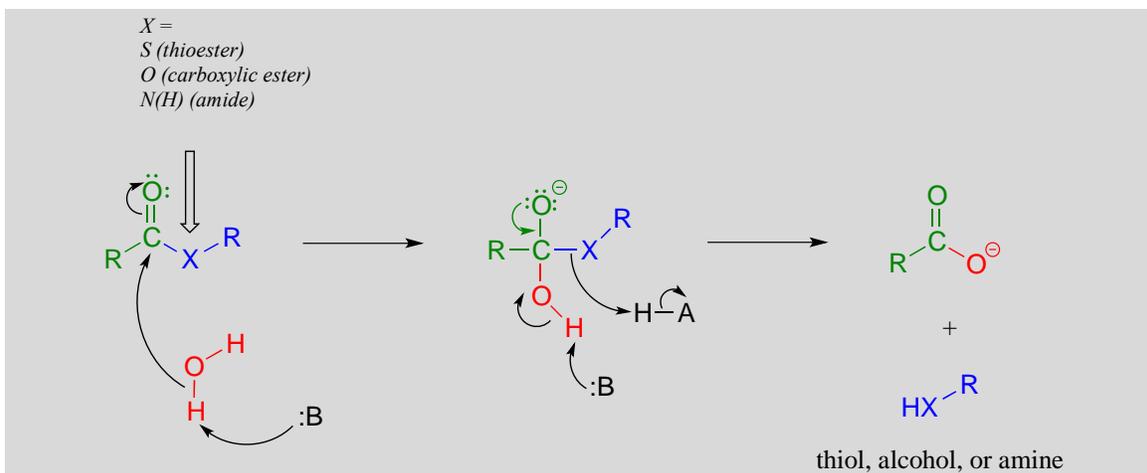
Section 11.6: Hydrolysis of thioesters, esters, and amides

So far we have been looking at the *formation* of thioesters, carboxylic esters, and amides, starting from carboxylates. In hydrolytic acyl substitution reactions, nucleophilic water is the incoming nucleophile and a carboxylate group is the final product. Because carboxylates are the least reactive among the carboxylic acid derivatives, these hydrolysis reactions are thermodynamically favorable, with thioester hydrolysis the most favorable of the three.

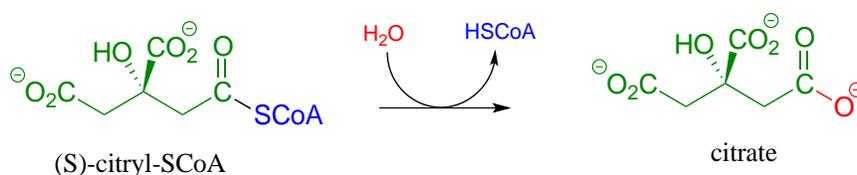
Thioester, carboxylic ester, and amide hydrolysis:



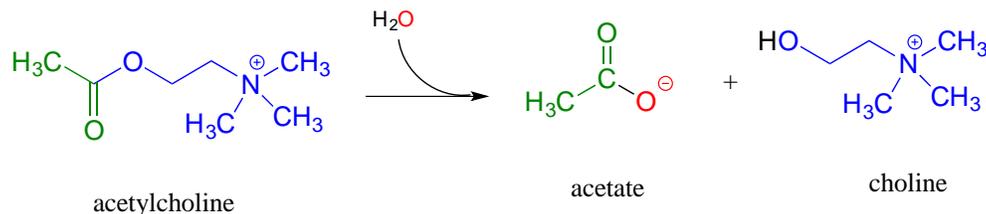
Mechanism:



In the citric acid (Krebs) cycle, (S)-citryl CoA is hydrolyzed to citrate (EC 2.3.3.8):



Acetylcholinesterase (EC 3.1.1.7), an enzyme present in the synapse, catalyzes hydrolysis of the ester group in acetylcholine, a neurotransmitter that triggers muscle contraction.

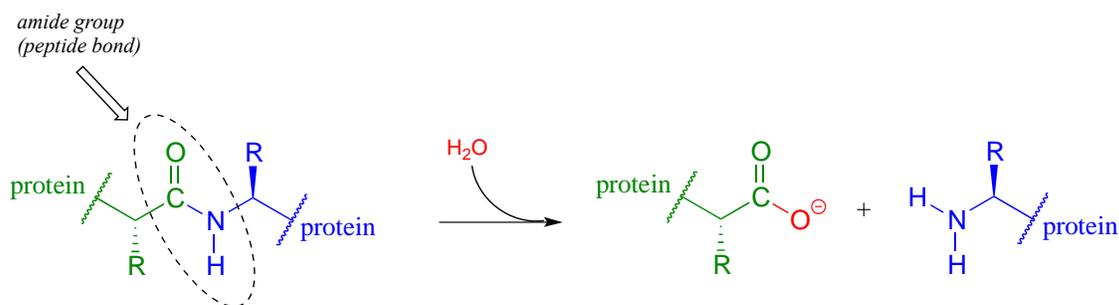


Like many other hydrolytic enzymes, the acetylcholinesterase reaction proceeds in two phases: first, a covalent enzyme-substrate intermediate is formed when the acyl group of acetylcholine is transferred to an active-site serine on the enzyme (a transesterification reaction). A water nucleophile then attacks this ester, driving off acetate and completing the hydrolysis.

Exercise 11.9: Based on the above description, draw the structure of the covalent enzyme-substrate intermediate in the acetylcholinesterase reaction.

If the action of acetylcholinesterase is inhibited, acetylcholine in the synapse does not get hydrolyzed and thus accumulates, resulting in paralysis and death in severe cases. **Sarin** nerve gas is a potent inhibitor of acetylcholinesterase action. Some victims of the Tokyo subway sarin attack in 1995 who were exposed to low levels of the gas reported that they initially realized that something was wrong when they noticed how dark everything seemed around them. This was due to uncontrolled contraction of their pupils. You will be invited to consider the mechanism of inhibition by sarin in problem 11.12.

Peptide (amide) bonds in proteins and polypeptides are subject to spontaneous (nonenzymatic) hydrolysis in water.



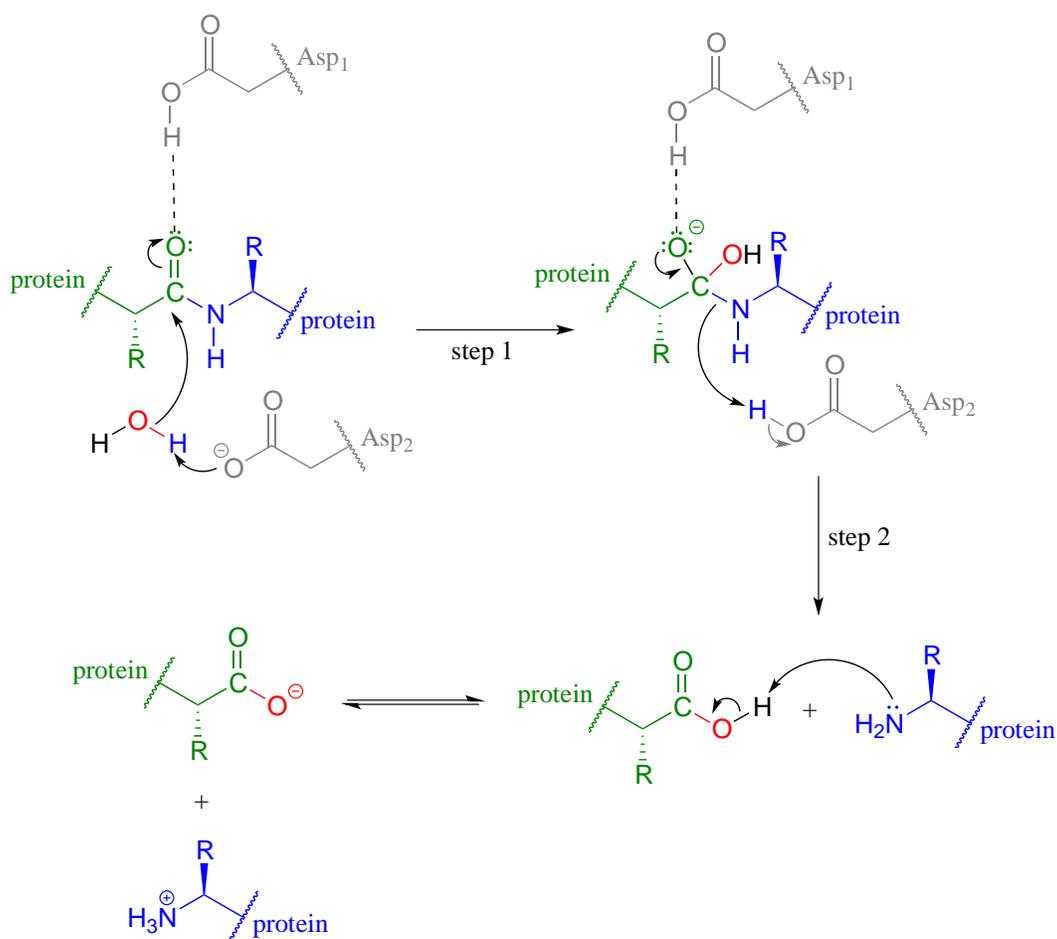
Although this amide to carboxylate conversion is *thermodynamically* a downhill reaction, peptide bonds are *kinetically* very stable (they react slowly) at neutral pH. In fact, the half-life for uncatalyzed hydrolysis of a peptide bond in pH 7 water is by some estimates as high as 1000 years.

The stability of peptide bonds makes good physiological sense: we would all be in trouble if our enzymes, receptors, and structural proteins were hydrolyzing away while we slept! That being said, it is also true that *controlled, specific* hydrolysis of peptide bonds, catalyzed by a large, diverse class of enzymes called **proteases**, is a critical biochemical reaction type that can occur very rapidly, in many different biological contexts. For example, many proteins only become active after they have been 'processed' - in other words, hydrolyzed at a specific amino acid location by a specific protease.

Although all proteases catalyze essentially the same reaction – amide hydrolysis - different protease subfamilies have evolved different catalytic strategies to accomplish the same result. **HIV protease** is the target of some of the most recently-developed anti-HIV drugs. It plays a critical role in the life cycle of the HIV virus, hydrolyzing specific peptide bonds of essential viral proteins in order to convert them to their active forms. HIV protease is a member of the **aspartyl protease** subfamily, so-named because of the

two aspartate residues located in the active sites of these enzymes. HIV protease is also, as you are probably aware, the target of **HIV protease inhibitor** drugs, which are a component of the most effective treatment currently available for HIV infection.

In HIV protease and other aspartyl proteases, the two enzymatic aspartates residues (shaded grey and abbreviated 'Asp₁' and 'Asp₂' in the figure below) work in concert to activate the electrophile, nucleophile, and leaving group in the reaction.

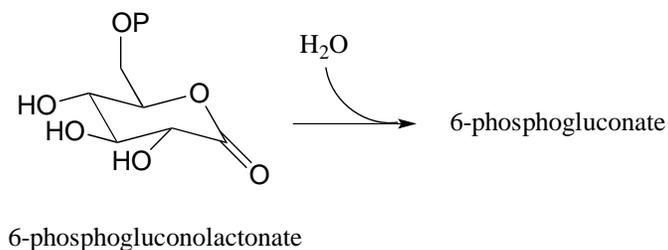


Exactly how this works is a subject of some debate and the details may well vary according to the enzyme in question, but one likely mechanism is illustrated in the figure above, where Asp₁, which is initially in its protonated form, contributes a hydrogen bond to draw electron density away from the carbonyl carbon, making it more electrophilic. At the same time, Asp₂, which begins the reaction cycle in its anionic form, deprotonates the water molecule to make it more nucleophilic. In step 2, Asp₂ donates a proton back to the nitrogen, making it a better leaving group.

HIV protease inhibitors shut down this reaction, which prevents the virus from processing the proteins that it uses to bond to host cells.

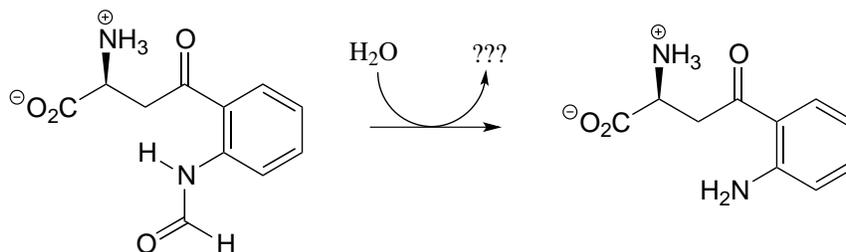
[Animation of HIV protease and inhibitors](#)

Exercise 11.10: Lactonase (EC 3.1.1.17), the second enzyme in the oxidative branch of the pentose phosphate pathway, catalyzes hydrolysis of the lactone (cyclic ester) group in 6-phosphogluconolactone. Draw the structure of 6-phosphogluconate, the product of this reaction.



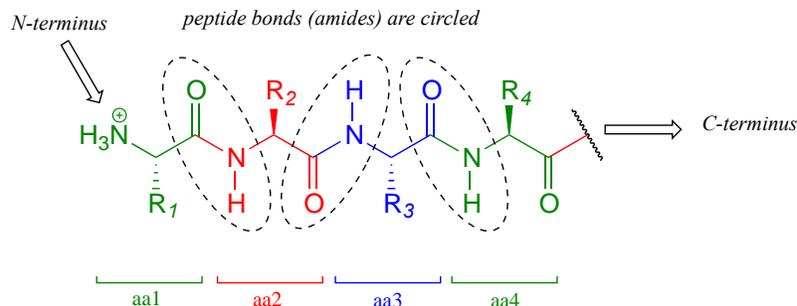
Exercise 11.11: Draw the product of the β -lactamase-catalyzed hydrolysis of penicillin as described in section 11.6.

Exercise 11.12: What is the missing product (designated below by question marks) in the reaction below, which is part of degradation pathway for the amino acid tryptophan? How could you describe this reaction in organic chemistry terminology?



Section 11.7: Protein synthesis on the ribosome

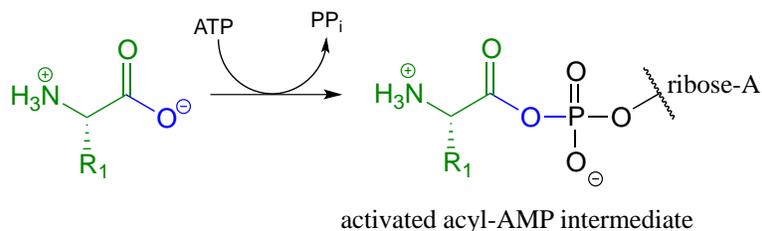
Recall from section 1.3D that the 'peptide bonds' which link amino acids to form polypeptides and proteins are in fact amide functional groups. The figure below shows the first four amino acid residues in a protein, starting at the amino terminus.



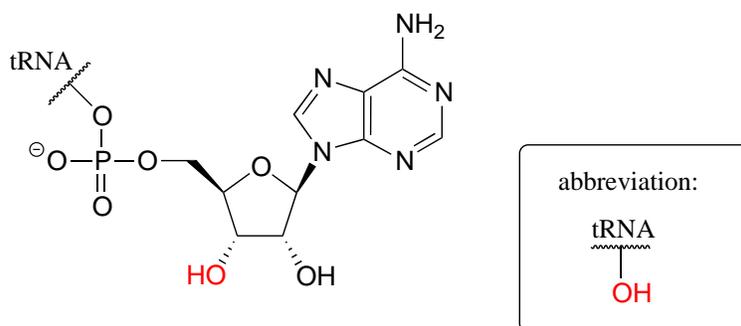
Let's take a look at the chemistry behind the formation of a new peptide bond between the first two amino acids - which we will call aa-1 and aa-2 - in a growing protein molecule. This process takes place on the ribosome, which is essentially a large biochemical 'factory' in the cell, composed up of many enzymes and RNA molecules, and dedicated to the assembly of proteins. You will learn more in a biochemistry or cell biology course about the complex but fascinating process of ribosomal protein synthesis. For now, we will concentrate on the enzyme-catalyzed organic transformation that is taking place: the formation of an amide from a carboxylate and an amine.

We have seen amide-forming reactions before – think back to the glutamine and asparagine synthetase reactions (section 11.5C). The same ideas that we learned for those reactions hold true for peptide bond formation: the carboxylate group on a substrate amino acid must first be activated, and the energy for this activation comes from ATP.

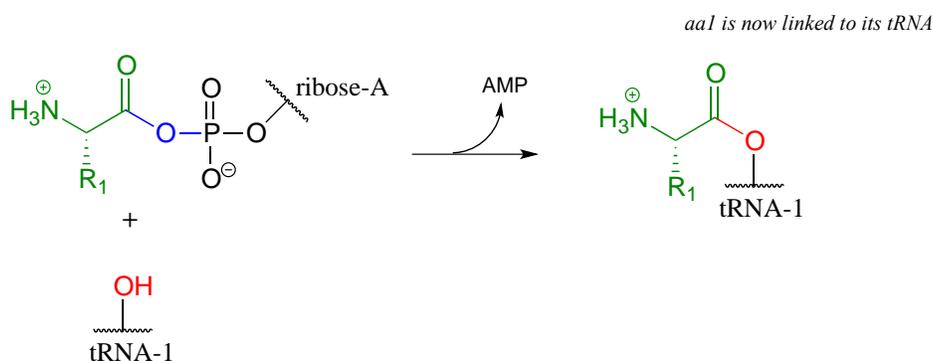
The carboxylate group of aa-1 is first transformed to an acyl-AMP intermediate through a nucleophilic substitution reaction at the α -phosphate of ATP.



In the next step, the amino acid is transferred to a special kind of RNA polymer called transfer RNA, or tRNA for short. We need not concern ourselves here with the structure of tRNA molecules- all we need to know for now is that the nucleophile in this reaction is a hydroxyl group on the terminal adenosine of a tRNA molecule. Because this tRNA molecule is specific to aa-1, we will call it tRNA-1

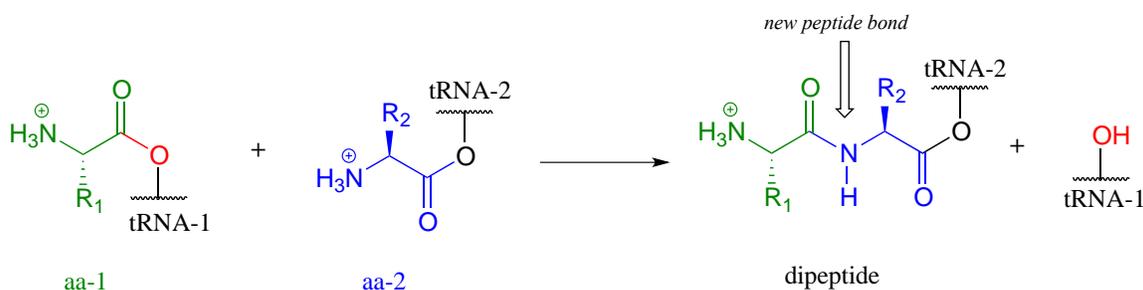


The incoming nucleophile is an alcohol, thus what we are seeing is an esterification: an acyl substitution reaction between the activated carboxylate of aa-1 and an alcohol on tRNA-1 to form an ester.

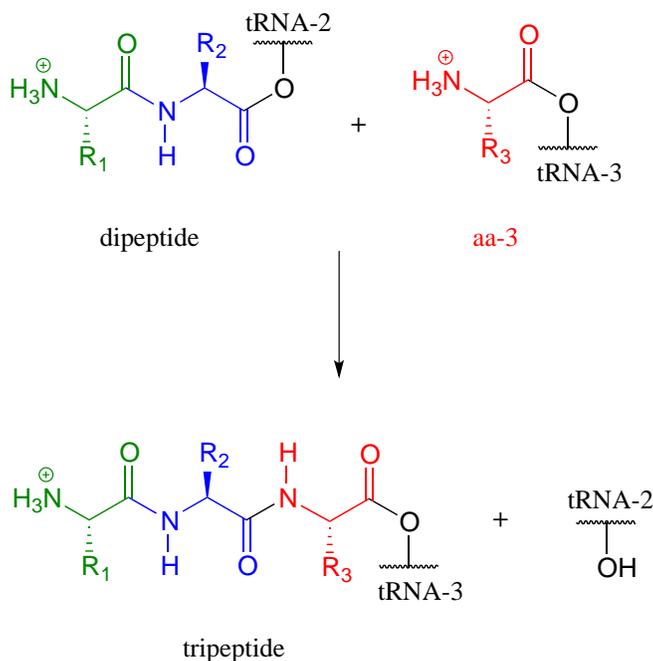


This reaction, starting with activation of the amino acid, is catalyzed by a class of enzymes called **aminoacyl-tRNA synthetases** (there are many such enzymes in the cell, each one recognizing its own amino acid - tRNA pair).

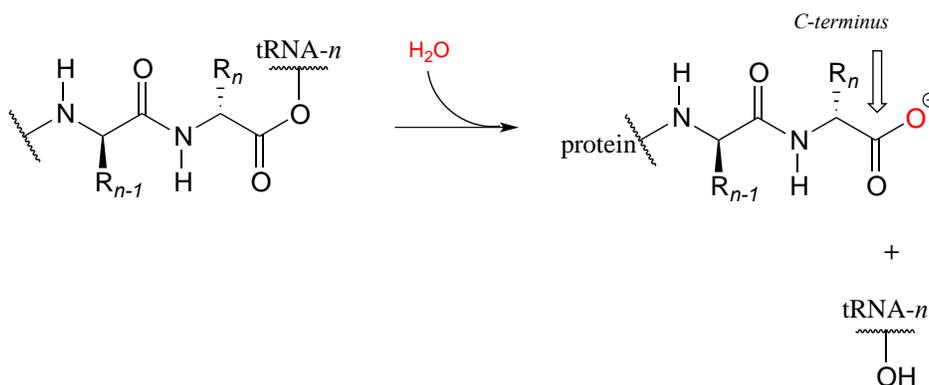
The first amino acid is now linked via an ester group to tRNA-1. The actual peptide bond-forming reaction occurs when a second amino acid (aa-2) also linked to its own tRNA-2 molecule, is positioned next to the first amino acid on the ribosome. In another acyl substitution reaction, catalyzed by an enzymatic component of the ribosome called **peptidyl transferase** (EC 2.3.2.12), the amino group on aa-2 displaces tRNA1: thus, an ester has been converted to an amide (thermodynamically downhill, so ATP is not required).



This process continues on the ribosome, as one amino acid after another is added to the growing protein chain:



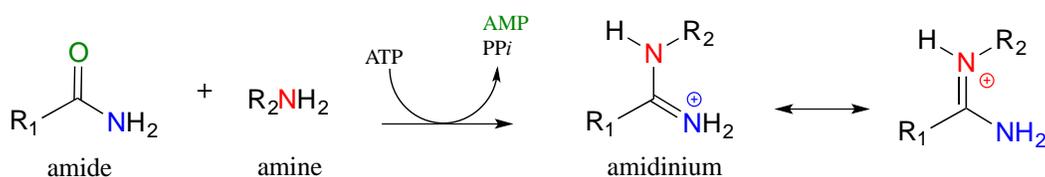
When a genetically-coded signal indicates that the chain is complete, an *ester hydrolysis* reaction – as opposed to another amide formation - occurs on the last amino acid, which we will call aa-*n*. This reaction is catalyzed by proteins called **release factors** (RFs).



This hydrolysis event frees the mature protein from the ribosome, and results in the formation of a free carboxylate group at the end of the protein (this is called the carboxy-terminus, or *C-terminus* of the protein, while the other end – the ‘starting’ end – is called the *N-terminus*).

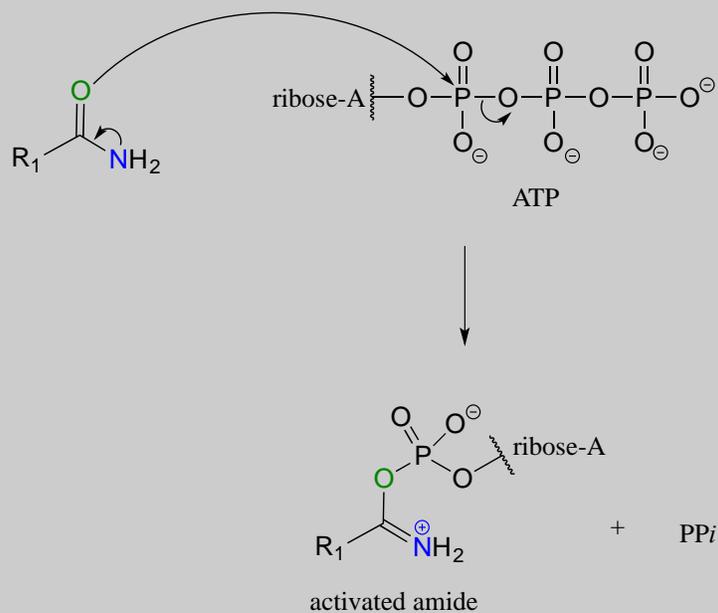
Section 11.8: Nucleophilic substitution at activated amides and carbamides

In discussing the nucleophilic acyl substitution reactions of acyl phosphates, thioesters, esters, and amides, we have seen many slight variations on one overarching mechanistic theme. Let's now look at a reaction that can be thought of as a 'cousin' of the nucleophilic acyl substitution, one that follows the same general pattern but differs in several details. Below is a generic illustration of this reaction type:



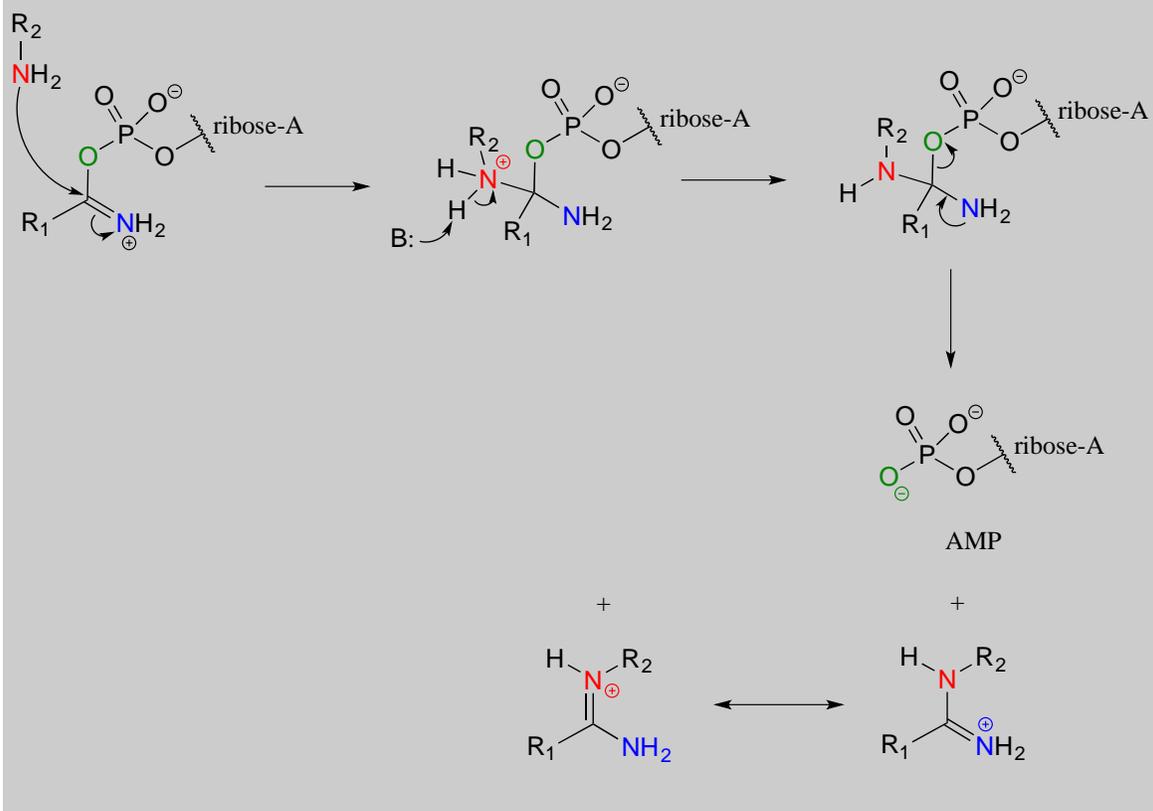
Looking at this reaction, you can see that substitution is occurring at an amide group, but the atom that gets expelled is the amide *oxygen* rather than the amide nitrogen. Also, we see that the substituting nucleophile is an amine, and the product is a functional group referred to as an **amidinium** ion (the uncharged conjugate base is called an **amidine**, and the pKa of the group is close enough to 7 that it can be shown in either protonated or deprotonated form in a biological context). As we learned previously in this chapter, amides are comparatively stable to nucleophilic substitution, and thus it stands to reason that the starting amide must be *activated* before the reaction can take place. This occurs, as you might predict, through the formation of an acyl phosphate intermediate at the expense of one ATP molecule. The amide *oxygen* acts as a nucleophile, attacking the α -phosphate of ATP to form the activated acyl-AMP intermediate:

Substitution at an activated amide, phase 1 (activation):

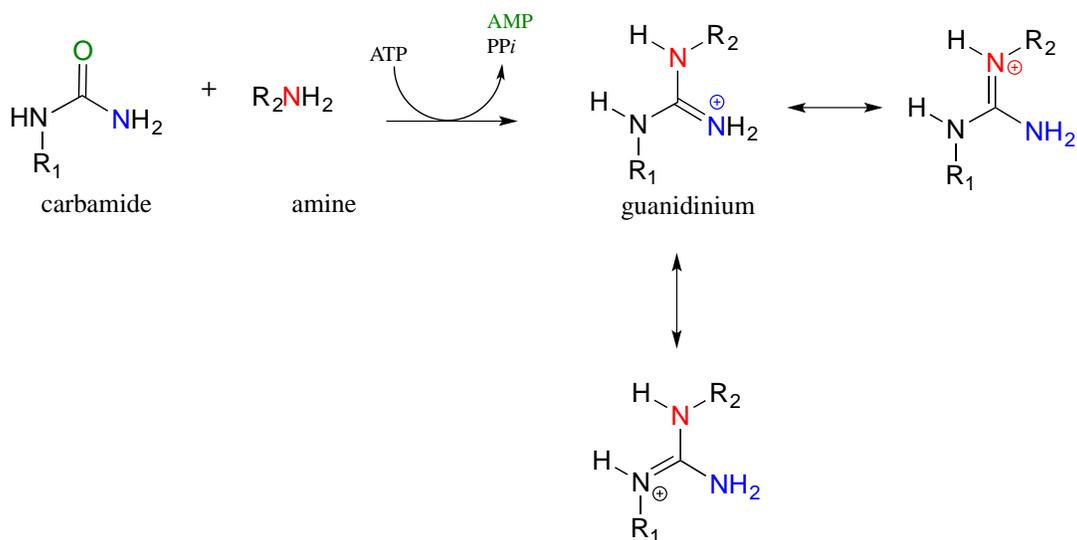


Next, a kind of acyl substitution occurs that we have not yet seen: an amine nitrogen attacks the electrophilic carbon of a carbon-*nitrogen* double bond, and the reaction proceeds through a tetrahedral intermediate before the AMP group is expelled, taking with it what was originally the carbonyl oxygen of the starting amide.

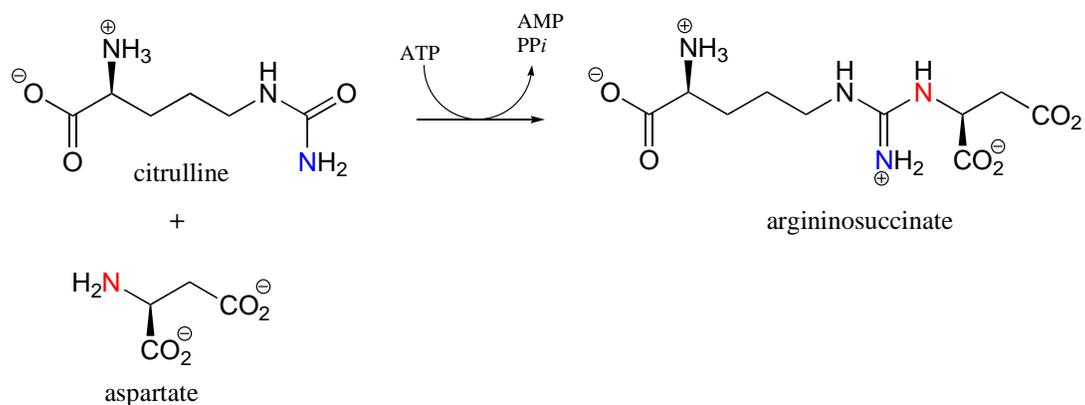
Substitution at an activated amide, phase 2:



Carbamides, a kind of 'double amide' functional group in which a carbonyl oxygen is bonded to *two* nitrogens, also undergo this type of reaction, leading to the formation of a **guanidinium/gaunidine** functional group (look again at the structure of the amino acid [arginine](#) and you will see that it contains a guanidinium group on the side chain).



The reaction catalyzed by argininosuccinate synthetase (EC 6.3.4.5) is the second step in the urea cycle, a four-step series of reactions in which ammonia is converted into urea for elimination in urine. Note that the substitution takes place at the carbamide group of citrulline, the nucleophilic amine is on aspartate, and the product has a guanidinium functional group.



Exercise 11.13

- Draw the structure of the 'activated carbamide' intermediate in the reaction above.
 - Draw the structure of the cyclic product of a *hypothetical* intramolecular substitution reaction of citrulline (ie. aspartate is not involved).
-

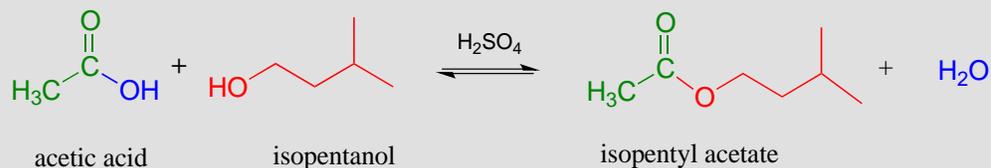
Section 11.9: Nucleophilic acyl substitution reactions in the lab

All of the biological nucleophilic acyl substitution reactions we have seen so far have counterparts in laboratory organic synthesis. Mechanistically, one of the biggest differences between the biological and the lab versions is that the lab reactions usually are run with a strong acid or base as a catalyst, whereas biological reactions are of course taking place at physiological pH. When proposing mechanisms, then, care must be taken to draw intermediates in their reasonable protonation states: for example, a hydronium ion (H_3O^+) intermediate is reasonable to propose in an acidic reaction, but a hydroxide (OH^-) intermediate is not.

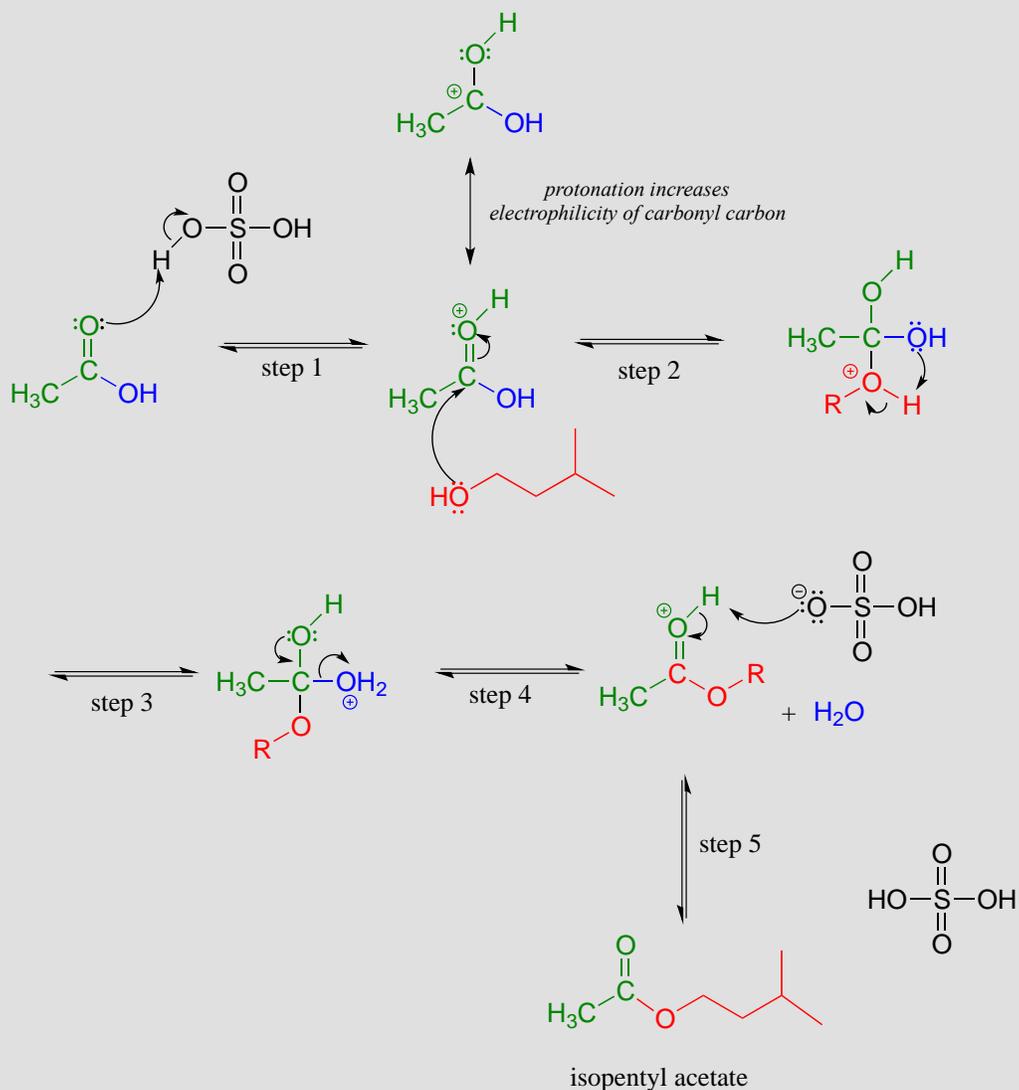
11.9A: Ester reactions - bananas, soap and biodiesel

Acid-catalyzed synthesis of flavor compounds such as isopentyl acetate (an ester with the flavor of banana) is simple to carry out in the lab. In this esterification reaction, acetic acid is combined with isopentyl alcohol along with a catalytic amount of sulfuric acid.

Acid-catalyzed esterification (laboratory reaction):



Mechanism:

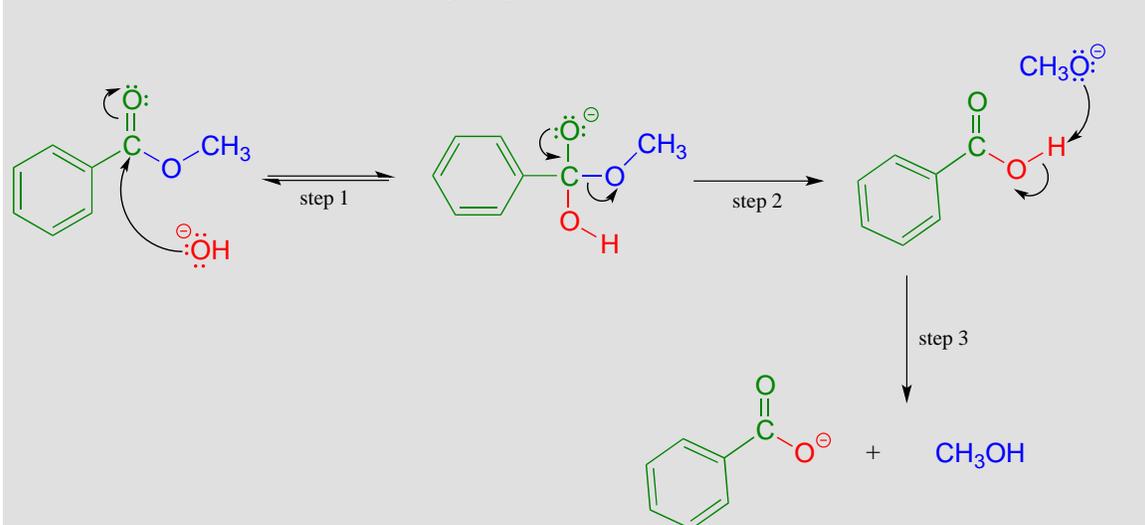


The carbonyl oxygen of acetic acid is first protonated (step 1), which draws electron density away from the carbon and increases its electrophilicity. In step 2, the alcohol nucleophile attacks: notice that under acidic conditions, the nucleophile is not deprotonated simultaneously as it attacks (as we would show in a biochemical mechanism), and the tetrahedral intermediate is a cation rather than an anion. In step 3, a proton is transferred from one oxygen atom to another, creating a good leaving group (water) which is expelled in step 4. Finally (step 5), the carbonyl oxygen on the ester is deprotonated, regenerating the catalytic acid.

This reaction is highly reversible, because carboxylic acids are approximately as reactive as esters. In order to obtain good yields of the ester, an excess of acetic acid can be used, which by Le Chatelier's principle (see your General Chemistry textbook for a review) shifts the equilibrium toward the ester product.

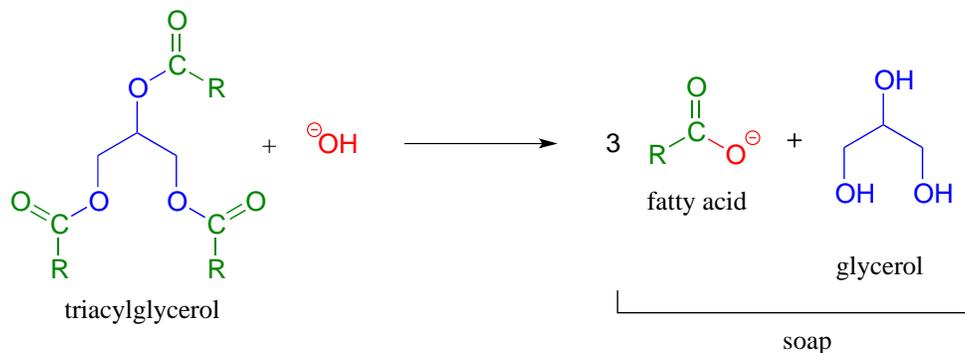
Saponification is a common term for base-induced hydrolysis of an ester. For example, methyl benzoate will hydrolyze to benzoate and methanol when added to water with a catalytic amount of sodium hydroxide.

Mechanism of base-catalyzed ester hydrolysis (saponification):

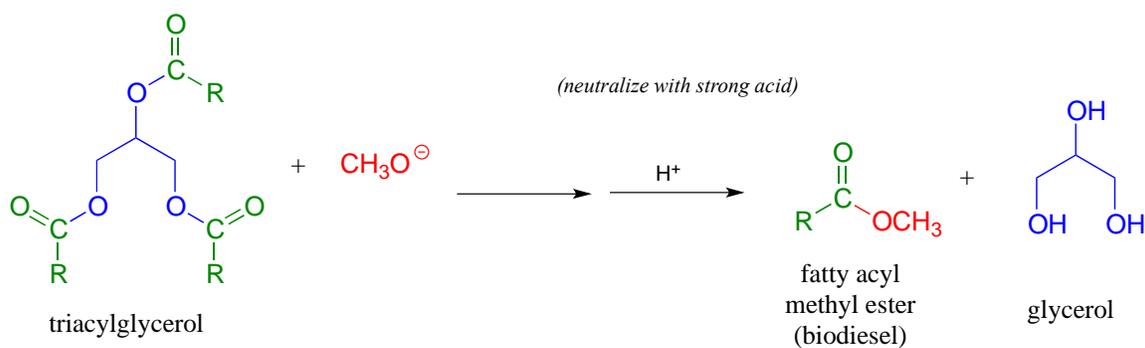


Addition of the base provides hydroxide ion to act as a nucleophile (hydroxide is of course a better nucleophile than water) in step 1. The tetrahedral intermediate (anionic in this case, because the reaction conditions are basic) then collapses in step 2, and the alkoxide (CH_3O^-) leaves. We are not used to seeing alkoxides or hydroxides as leaving groups in biochemical reactions, because they are strong bases - but in a basic solution, this is a reasonable chemical step. Step 3 is simply an acid-base reaction between the carboxylic acid and the alkoxide. Note that this is referred to as base-*induced* rather than base-catalyzed because hydroxide is not regenerated, and thus a full molar equivalent of base must be used.

The saponification process derives its name from the ancient craft of soap-making, in which the ester groups of triacylglycerols in animal fats are hydrolyzed under basic conditions to glycerol and fatty acyl anions (see section 2.5A for a reminder of how fatty acyl anions work as soap).



We learned earlier about transesterification reactions in the context of the chemical mechanism of aspirin. Transesterification also plays a key role in a technology that is already an important component in the overall effort to develop environmentally friendly, renewable energy sources: biodiesel. You may have heard stories about people running their cars on biodiesel from used french fry oil. To make biodiesel, triacylglycerols in fats and oils can be transesterified with methanol or ethanol under basic conditions. The fatty acyl methyl and ethyl ester products are viable motor fuels.



Exercise 11.14: Draw structures of the carboxylic acid and alcohol starting materials that could be used to synthesize the fragrant fruit esters shown in section 11.1.

Exercise 11.15: What would happen if you tried to synthesize isopentyl acetate (banana oil) with basic rather than acidic conditions? Would this work?

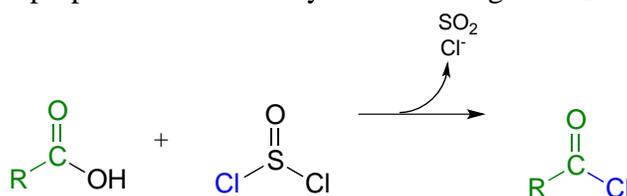
Exercise 11.16: Consider the reverse direction of the acid-catalyzed esterification reaction. What would you call this reaction in organic chemistry terms?

Exercise 11.17: An alternative way to synthesize esters is to start with a carboxylate and an alkyl halide. Draw a mechanism for such a synthesis of methyl benzoate - what type of reaction mechanism is this?

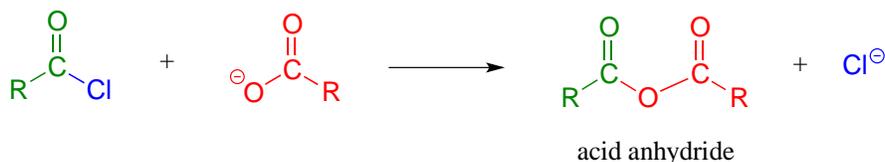
11.9B: Acid chlorides and acid anhydrides

In the cell, acyl phosphates and thioesters are the most reactive of the carboxylic acid derivatives. In the organic synthesis lab, their counterparts are acid chlorides and acid anhydrides, respectively. Of the two, acid chlorides are the more reactive, because the chloride ion is a weaker base and better leaving group than the carboxylate ion (the pK_a of HCl is -7, while that of carboxylic acids is about 4.5: remember, a stronger conjugate acid means a weaker conjugate base).

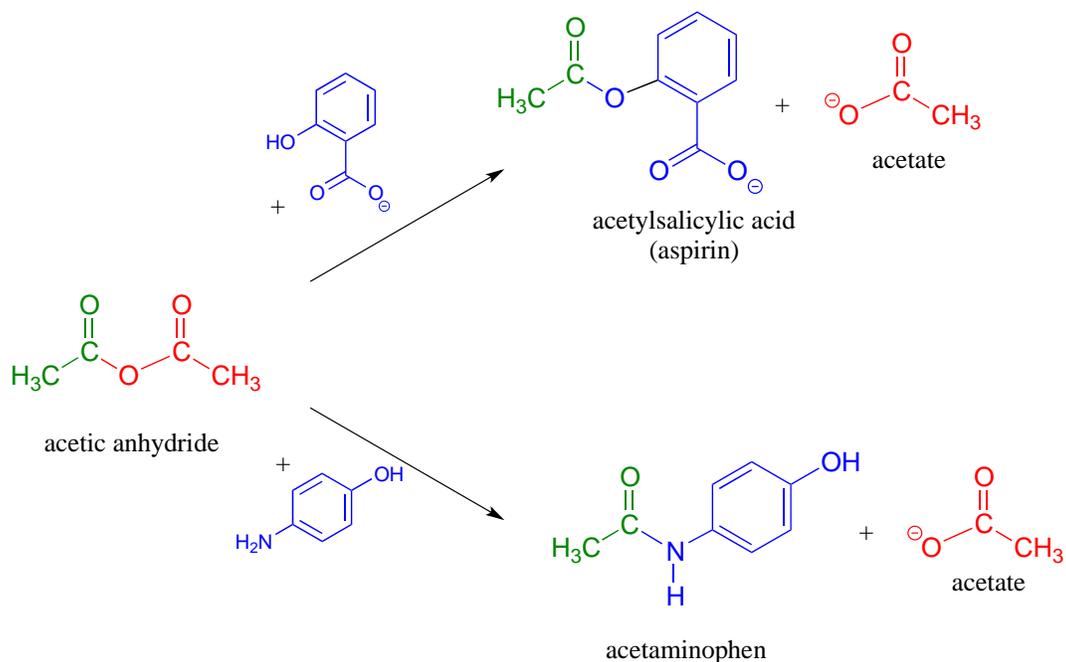
Acid chlorides can be prepared from carboxylic acids using SOCl₂:



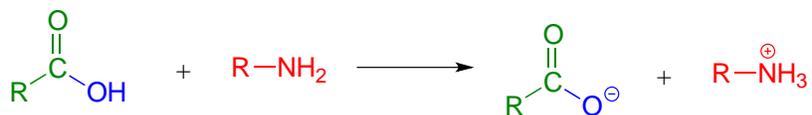
Acid anhydrides can be prepared from carboxylic acids and an acid chloride under basic conditions:



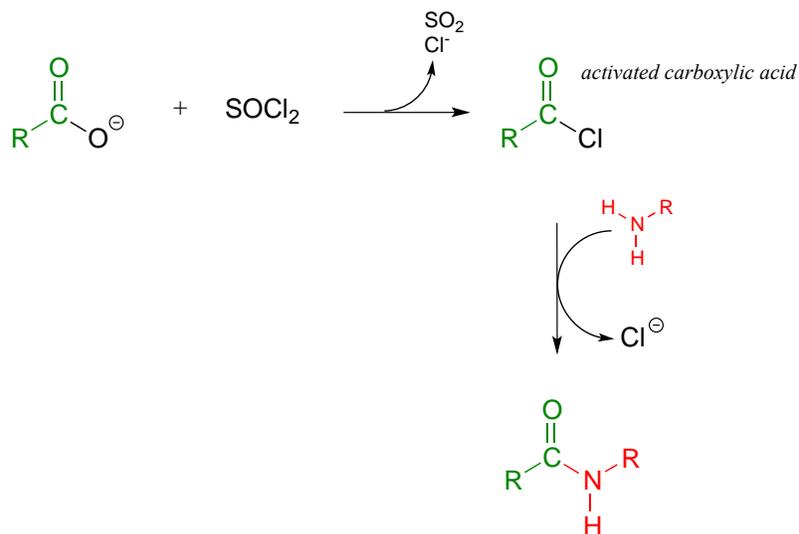
Acetic anhydride is often used to prepare acetate esters and amides from alcohols and amines, respectively. The synthesis of aspirin and acetaminophen are examples:



A carboxylic acid cannot be *directly* converted into an amide because the amine nucleophile would simply act as a base and deprotonate the carboxylic acid:

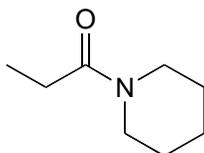


Instead, the carboxylic acid is first converted to an acid chloride (in other words, the carboxylic acid is *activated*), then the acid chloride is combined with an amine to make the amide.



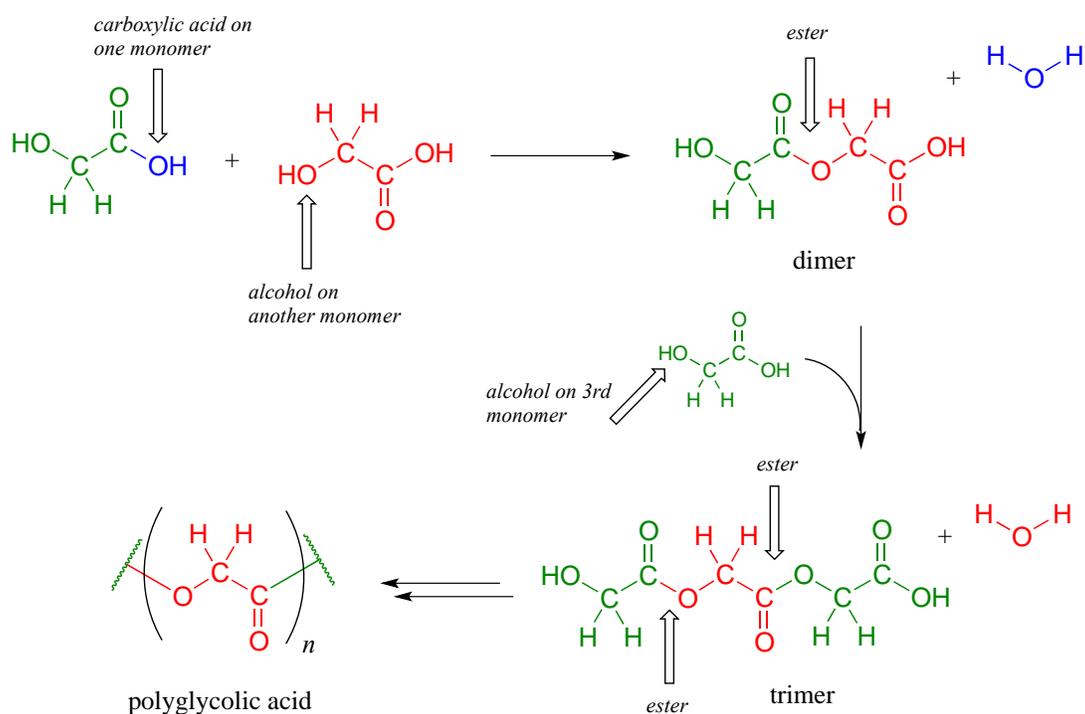
This sequence of reactions is a direct parallel to the biochemical glutamine and asparagine synthase reactions we saw earlier in the chapter (section 11.5A), except that the activated form of carboxylic acid is an acid chloride instead of an acyl phosphate or acyl-AMP.

Exercise 11.18: For the preparation of the amide below, show a starting carboxylate and amine and the intermediate acid chloride species.



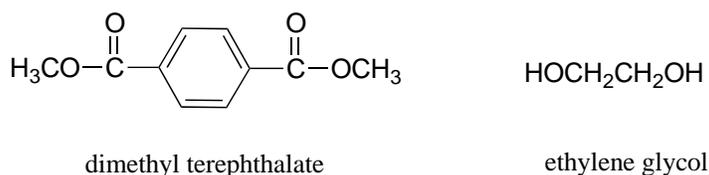
11.9C: Polyesters and polyamides

If you have ever had the misfortune of undergoing surgery or having to be stitched up after a bad cut, it is likely that you benefited from our increasing understanding of polymers and carboxylic ester chemistry. Polyglycolic acid is a material commonly used to make dissolving sutures. It is a **polyester** - a polymer linked together by ester groups - and is formed from successive acyl substitution reactions between the alcohol group on one end of a glycolic acid monomer and the carboxylic acid group on a second:



The resulting polymer - in which each strand is generally several hundred to a few thousand monomers long - is strong, flexible, and not irritating to body tissues. It is not, however, permanent: the ester groups are reactive to gradual, spontaneous hydrolysis at physiological pH, which means that the threads will dissolve naturally over several weeks, eliminating the need for them to be cut out by a doctor.

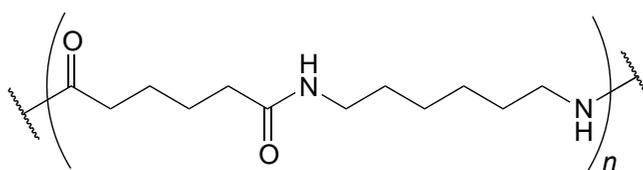
Exercise 11.19: Dacron, a polyester used in clothing fiber, is made of *alternating* dimethyl terephthalate and ethylene glycol monomers.



a) Draw the structure of a Dacron tetramer (in other words, four monomers linked together).

b) Water is a side product of glycolic acid polymerization. What is the equivalent side product in Dacron production?

Exercise 11.20: Nylon 6,6 is a widely used polyamide composed of alternating monomers. Nylon 6,6 has the structure shown below -the region within the parentheses is the repeating unit, with 'n' indicating a large number of repeats. Identify the two monomeric compounds used to make the polymer.

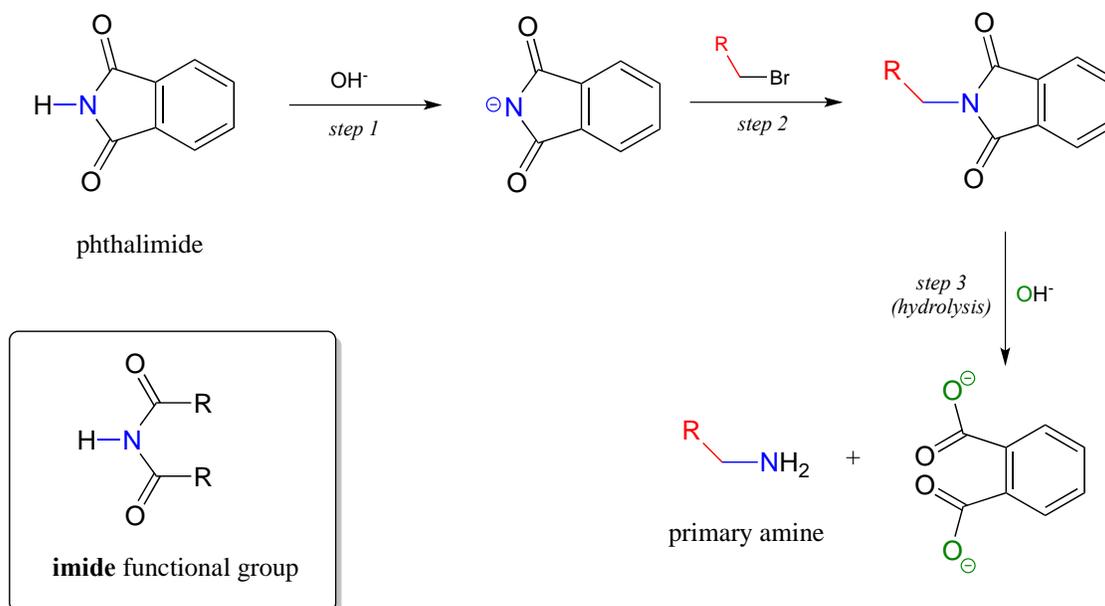


Nylon 66

11.9D: The Gabriel synthesis of primary amines

The Gabriel synthesis, named after the 19th-century German chemist Sigmund Gabriel, is a useful way to convert alkyl halides to amines and another example of S_N2 and acyl substitution steps in the laboratory. The nitrogen in the newly introduced amine group comes from phthalimide. In the first step of the reaction, phthalimide is deprotonated by

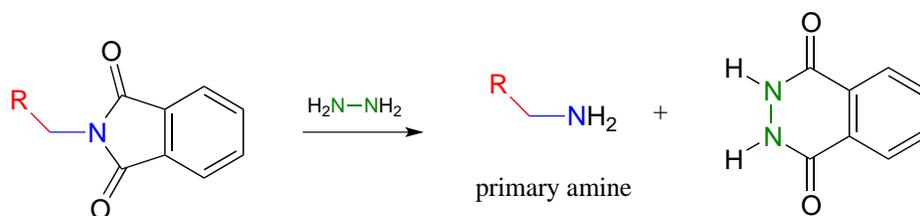
hydroxide, then in step 2 it acts as a nucleophile to displace a halide in an S_N2 reaction (phthalimide is not a very powerful nucleophile, so this reaction works only with unhindered primary or methyl halides).



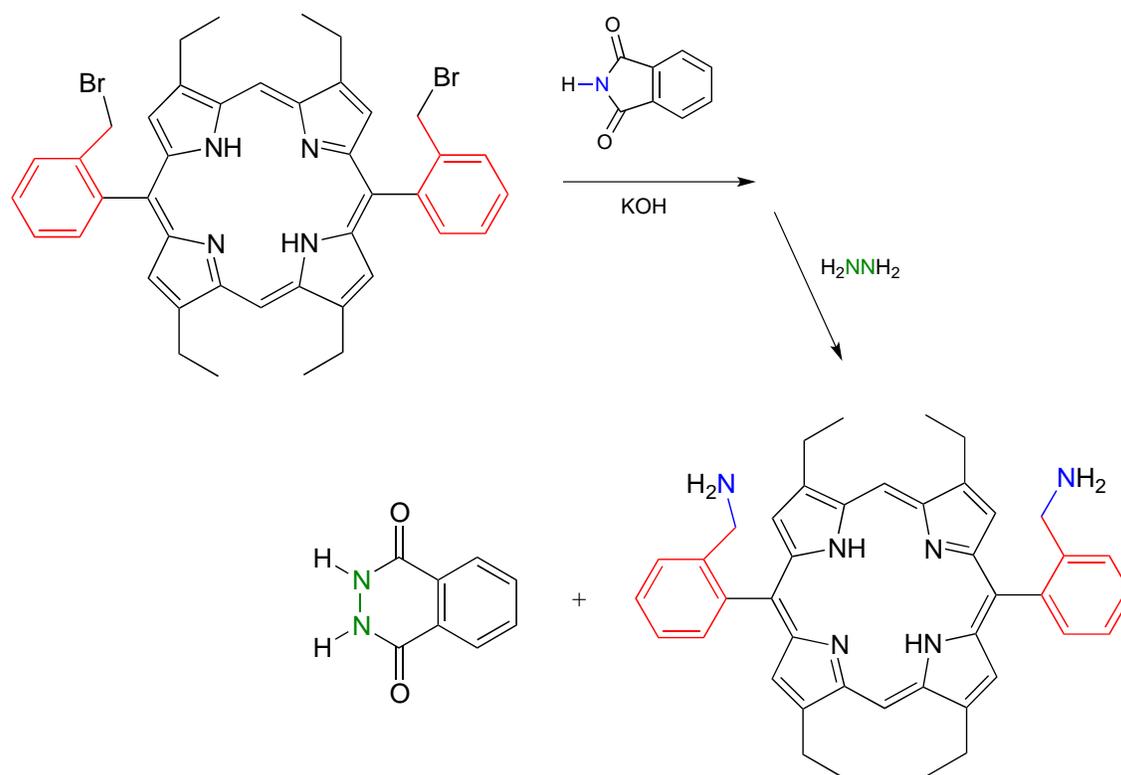
Step 3 is simply a pair of hydrolytic acyl substitution steps to release the primary amine, with an aromatic dicarboxylate by-product.

Exercise 11.21: Phthalimide contains an 'imide' functional group, and has a pK_a of approximately 10. What makes the imide group so much more acidic than an amide, which has a pK_a of approximately 17?

As an alternative procedure, release of the amine in step 3 can be carried out with hydrazine (H_2N-NH_2) instead of hydroxide. Again, this occurs through two nucleophilic acyl substitution reactions.



In 2000, chemists at MIT synthesizing a porphyrin-containing molecule introduced two amine groups using the Gabriel synthesis with hydrazine. Porphyrins, which include the 'heme' in our red blood cells, are an important family of biomolecules with a variety of biochemical function (*J. Org. Chem.* **2000**, 65, 5298).



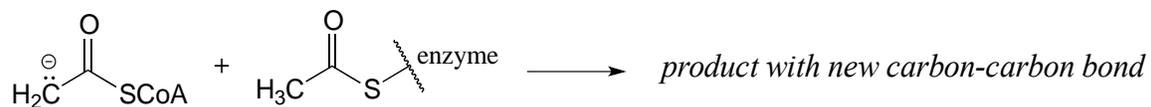
Section 11.10: A look ahead - acyl substitution reactions with a carbon or hydride nucleophile

Although we have seen many different types of nucleophilic acyl substitutions in this chapter, we have not yet encountered a reaction in which the incoming nucleophile is a carbanion or a hydride. Recall that in the previous chapter on aldehydes and ketones, we also postponed discussion of nucleophilic carbonyl addition reactions in which a carbanion or a hydride is the nucleophile. The reason for putting off these discussions is that these topics are both important and diverse enough to warrant their own dedicated chapters.

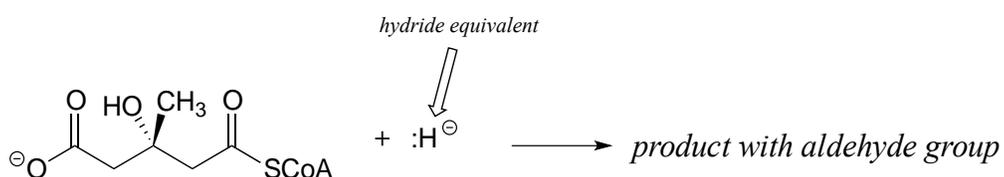
In the next chapter, we will see many carbonyl addition and acyl substitution reactions where the nucleophilic species is a resonance-stabilized *carbanion* such as an enolate (section 7.6B). Then in chapter 14, we will encounter nucleophilic addition and acyl substitution reactions in which a *hydride ion* (H⁻) essentially plays the part of a nucleophile. In these chapters we will see how nucleophilic carbanion and hydride species are generated in a biochemical context. For now, see if you can predict the result of the following biochemical reactions.

Exercise 11.22: Predict the products of the following nucleophilic acyl substitution reactions, both of which are part of the biosynthesis of isoprenoid compounds such as cholesterol and lycopene:

a) acetoacetyl CoA acetyltransferase reaction (enolate nucleophile)



b) HMG-CoA reductase reaction (to repeat, the nucleophile here is not *literally* an isolated hydride ion, which would be a very unlikely species in a physiological environment. We will learn in chapter 16 what is actually going on, but for the time being, just predict the result of an acyl substitution reaction with a "hydride ion" nucleophile.)



Key learning objectives for this chapter

Be able to recognize and draw examples of carboxylic acid derivative functional groups:

- carboxylic acids/carboxylates
- acyl phosphates (both acyl monophosphate and acyl-AMP)
- thioesters
- esters
- amides
- acid chlorides
- carboxylic acid anhydrides

Know the meaning of the terms 'acyl', 'acetyl', 'formyl', 'lactone', and 'lactam'.

You need not memorize the structure of coenzyme A, but you should recognize that it contains a key thiol group and often forms thioester linkages, particularly in fatty acid metabolism.

Understand what happens in a nucleophilic acyl substitution (also called acyl transfer reaction), and be able to draw mechanistic arrows for a generalized example.

Know the trends in relative reactivity for the carboxylic acid derivatives:

- in a biological context (acyl phosphates and thioesters as activated acyl groups)
- in a laboratory context (acid chlorides and carboxylic acid anhydrides as activated acyl groups)

Recognize and understand the most important types of nucleophilic acyl substitution reactions in biology:

- How a carboxylate group, which is unreactive to nucleophilic acyl substitution reactions, is activated in the cell by ATP-dependent phosphorylation to either acyl monophosphate or acyl-AMP.
- Conversion of an acyl phosphate to a thioester, a (carboxylic) ester, or an amide.
- Transthioesterification, esterification, and transesterification reactions.
- Conversion of a thioester or ester to an amide
- Hydrolysis of a thioester, a (carboxylic) ester, or an amide to a carboxylate.

Understand the energetics of the above reactions:

- Carboxylate to acyl phosphate is 'uphill' energetically, paid for by coupling to hydrolysis of one ATP

- Other conversions above are 'downhill': it is unlikely, for example, to see a direct conversion of an amide to an ester.

(Notable exception: the lactam (cyclic amide) group in penicillin is very reactive due to ring strain, and forms an ester with an active site serine residue in the target protein)

You need not memorize all of the details of peptide bond formation on the ribosome, but you should be able to follow the description in section 7 and recognize the nucleophilic acyl substitution reactions that are occurring.

Be able to draw complete mechanisms for the following lab reactions:

acid-catalyzed esterification of a carboxylic acid
saponification (base-catalyzed hydrolysis of an ester), application to soap-making
base-catalyzed transesterification, application to biodiesel production

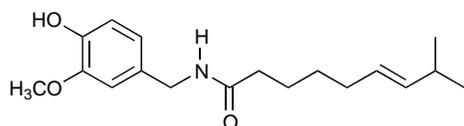
Understand how acid chlorides and carboxylic acid anhydrides serve as activated acyl groups in laboratory synthesis. Be able to describe how an amide to ester conversion could be carried out in the laboratory.

Understand how polyesters and polyamides are formed. Given the structure of a polymer be able to identify monomer(s), and vice-versa.

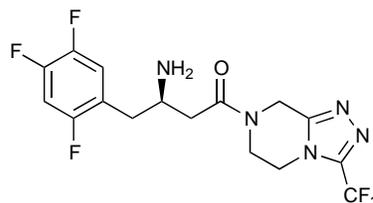
Be able to recognize, predict products of, and draw mechanisms for the Gabriel synthesis of primary amines, using either hydroxide ion or hydrazine to release the amine product.

Problems

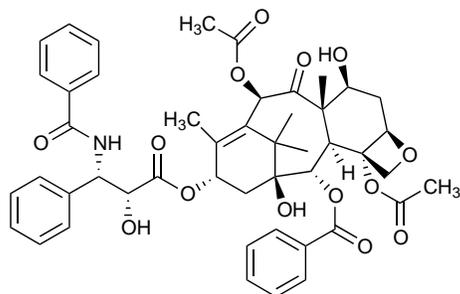
P11.1: Here is some practice in recognizing carboxylic acid derivative functional groups in large, complex biological molecules. There are seven amide and four ester groups in the molecules below - see if you can find them all.



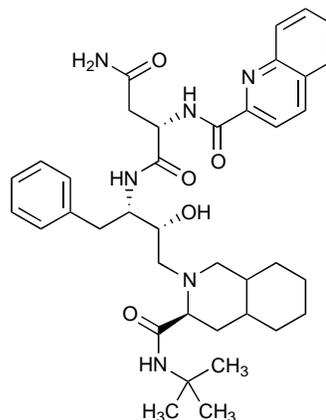
capsaicin
(the 'hot' molecule in hot peppers)



sitagliptin
(component of Janumet, a diabetes drug from Merck Pharmaceuticals)



paclitaxel
(cancer drug Taxol from Bristol-Myers Squibb)

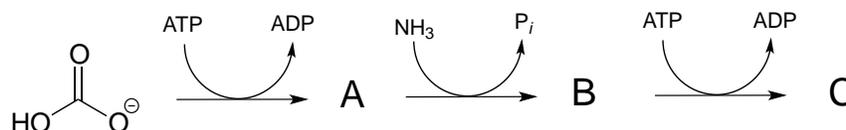


Saquinavir
(first HIV protease inhibitor to be approved by FDA)

P11.2

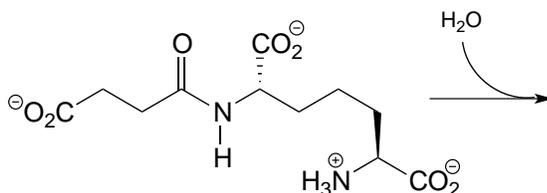
a) Predict the structures of intermediate compounds A, B, and C in the reaction below (EC 6.3.4.16). Compound C contains an activated carboxylate functionality. Use abbreviations as appropriate.

b) Draw a reasonable mechanism for the A to B step



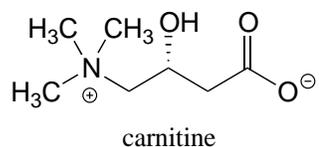
P11.3: Imagine that acetylcholine is combined with acetylcholinesterase (section 11.6) in a buffer made from ^{18}O -labeled water. Where would you expect to find the ^{18}O label in the products?

P11.4: Predict the products of this hydrolysis reaction (EC 3.5.1.18):



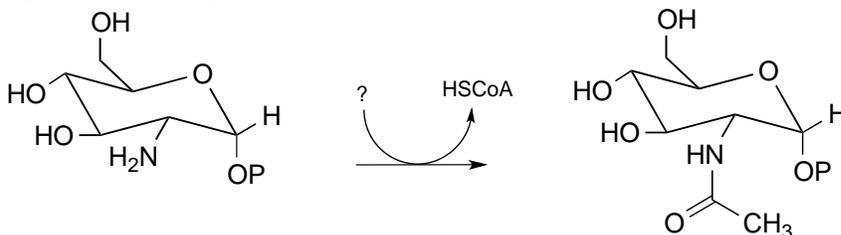
P11.5: The breakdown of fat in our bodies begins with the action of lipase enzymes, which catalyze the cleavage of fatty acids from the glycerol backbone of triacylglycerol (see section 1.3A for a reminder of the structure of triacylglycerol). A serine residue in the lipase active site plays a key nucleophilic role in the reaction. Draw the *single mechanistic step* in which the covalent link between a fatty acid and the glycerol backbone is broken, using curved arrow notation and appropriate abbreviation.

P11.6: Before long-chain fatty acids are transported across the inner mitochondrial membrane, they are temporarily transferred from Coenzyme A to a transport molecule called carnitine, to which they are linked by an ester group (EC 2.3.1.21).

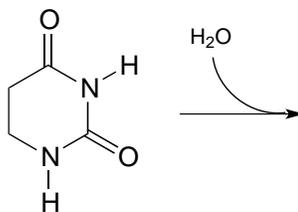


Draw the structure of fatty acyl carnitine (use R to denote the hydrocarbon chain of the fatty acid)

P11.7: Below is a reaction from carbohydrate metabolism (EC 2.3.1.157). Identify the compound designated with a question mark.



P11.8: Propose the most likely enzymatic hydrolysis product of the substrate below (hint - think about electrophilicity when considering regiochemical outcomes!) (EC 3.5.2.2)

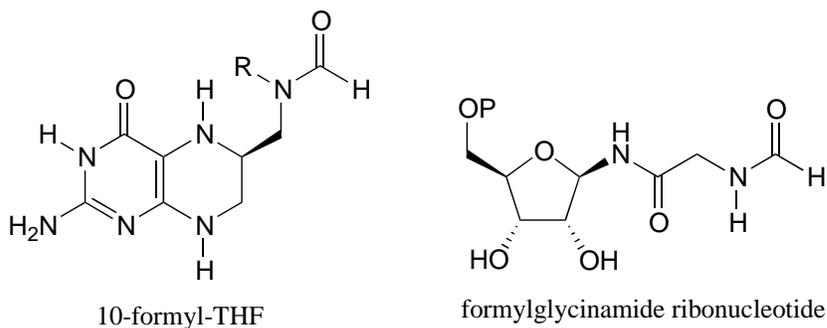


P11.9: The coenzyme tetrahydrofolate (THF) participates in single-carbon transfer reactions. One derivative of THF, called 10-formyl-THF (abbreviated structure shown below), transfers a formyl group early in purine ribonucleotide biosynthesis to glycinamide ribonucleotide.

10-formyl-THF + glycinamide ribonucleotide

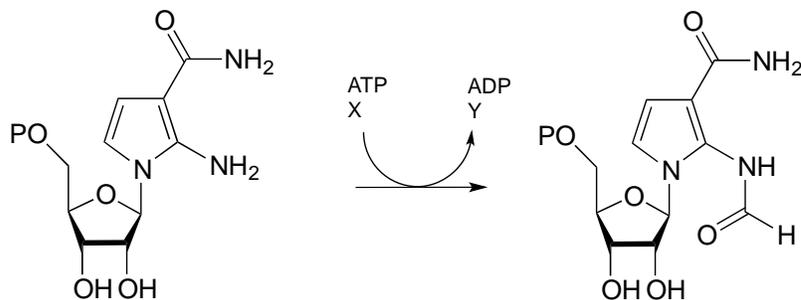


THF + formylglycinamide ribonucleotide



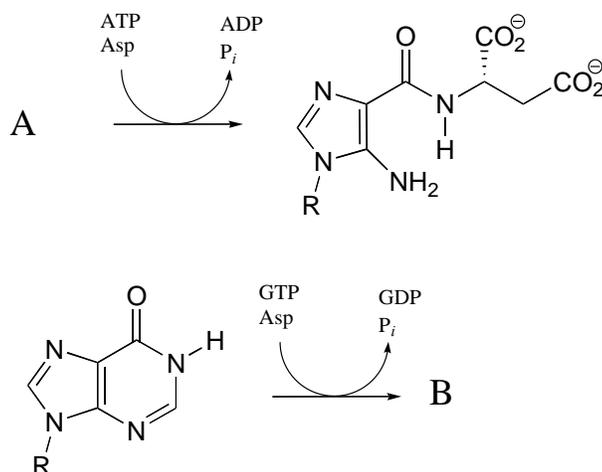
Draw a nucleophilic attack step for this reaction (assume that acyl transfer between the two substrates is direct, without any covalent enzyme-substrate intermediates being formed).

P11.10: One of the key steps in the biosynthesis of purine nucleotides (guanosine and adenosine) in archaea is shown below.

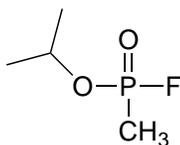


Identify the missing compounds X and Y in the figure above, and draw the structure of an acyl phosphate intermediate.

P11.11: The reactions below are part of nucleotide biosynthesis. Predict the structures of compounds A and B. Compound A contains a carboxylate group, and the reaction that forms compound B is of the type discussed in section 11.8, in which an amine group substitutes at an activated amide to form an amidine/amidinium group.

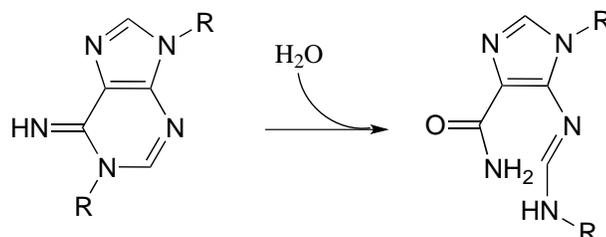


P11.12: Recall from section 11.6 that acetylcholinesterase catalyzes the hydrolysis of the ester group in acetylcholine, going through an intermediate in which the acetyl group on the substrate is transferred to a serine on the enzyme by a transesterification reaction. The nerve gas sarin acts by blocking this initial transesterification step: the drug enters the active site and attaches to the active site serine. Given the structure of sarin below, propose a mechanism for how this happens.

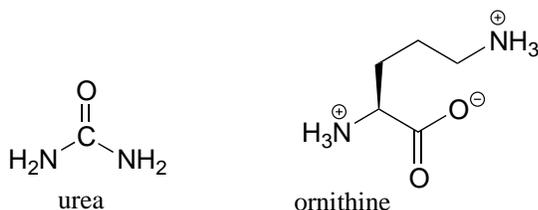


sarin

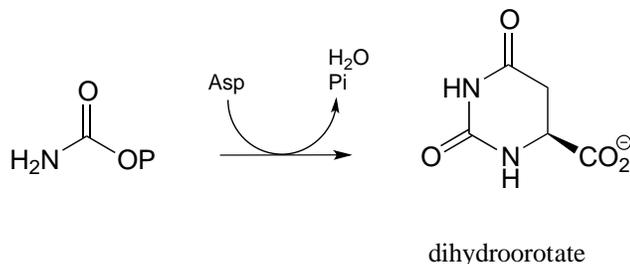
P11.13: Propose a mechanism for the following reaction from histidine biosynthesis (EC 3.5.4.19).



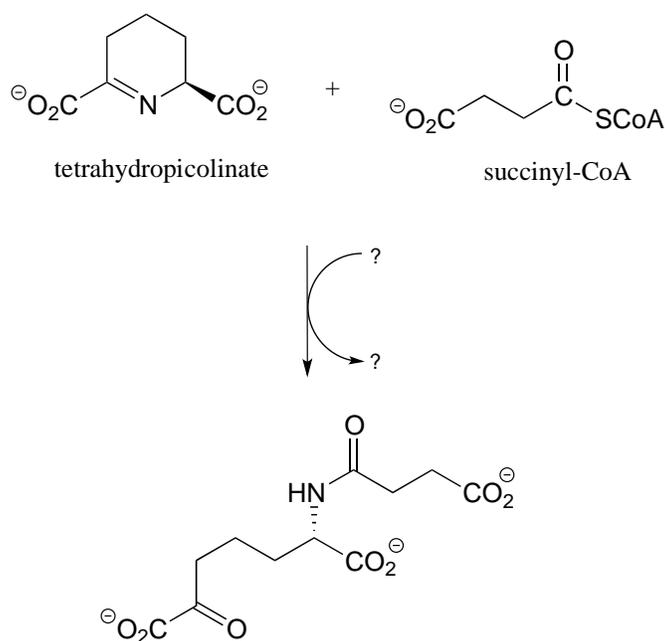
P11.14 (Challenging!) In the final step of the urea cycle (a phase of amino acid degradation pathways), the amino acid arginine is hydrolyzed to urea and ornithine (EC 3.5.3.1). Propose a reasonable mechanism.



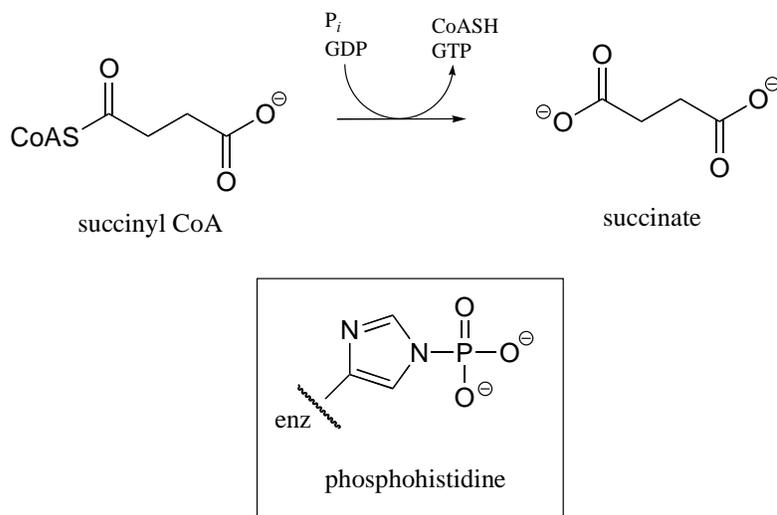
P11.15: In the biosynthetic pathway for the DNA/RNA bases uridine and cytidine, a reaction occurs in which carbamoyl phosphate condenses with aspartate, and the resulting intermediate cyclizes to form dihydroorotate. Propose a mechanism for this transformation. *Hint:* in a very unusual step, a carboxylate group is at one point in the process *directly* subjected to an acyl transfer reaction, without prior activation by phosphorylation. The enzyme accomplishes this with the help of two bound zinc ions, which serve to stabilize the negative charge on a hydroxide leaving group. (EC 3.5.2.3) (*Biochemistry* **2001**, 40, 6989, Scheme 2)



P11.16: Propose a reasonable mechanism for the reaction below (from lysine biosynthesis), and fill in the missing species indicated by question marks.

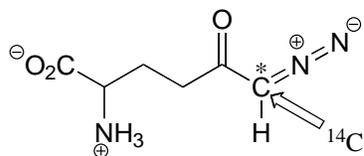


P11.17: In a step in the citric acid cycle, hydrolysis of succinyl-CoA is coupled to phosphorylation of GDP. The mechanism involves the transient phosphorylation of an active site histidine. Suggest a (multi-step) mechanism for this process (EC 6.2.1.4).



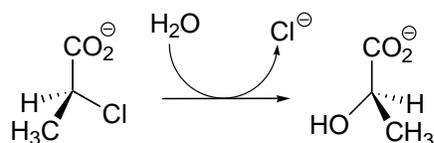
P11.18: A ^{14}C -labeled diazoketone compound (structure below) was used to irreversibly inactivate an enzyme called glutaminase A. Inactivation was shown to occur with ^{14}C labeling of an active site cysteine.

a) Propose a mechanism of inactivation and cysteine labeling.



b) To a lesser extent, inactivation of the enzyme and labeling of the cysteine was found to occur with release of a radioactive compound from the active site. Propose a mechanism for the mode of inactivation.

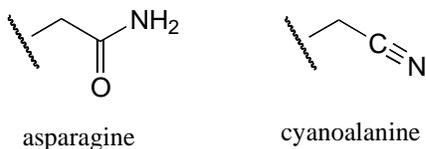
P11.19: Dehalogenase enzymes catalyze the cleavage of carbon-halogen bonds, and are of interest to scientists looking for new ways to detoxify organohalogen pollutants that make their way into the environment. One such dehalogenase catalyzes the following reaction:



An active site aspartate is thought to carry out the initial nucleophilic attack that expels the chloride.

a) Draw a likely mechanism for the complete reaction shown above. Look carefully at the stereochemical progress!

b) When the active site aspartate was mutated to asparagine, the enzyme still maintained activity. Mass spectrometry analysis indicated that, at one point in the catalytic cycle of the mutant enzyme, the asparagine exists as a cyanoalanine. Draw a likely mechanism for the reaction as catalyzed by the mutant enzyme, including formation of the transient cyanoalanine residue and subsequent regeneration of the asparagine.



Chapter 12

Reactions at the α -carbon, part I



photo credit: Alan Couch (<https://www.flickr.com/photos/couchy/>)

Introduction

There are lots of things that can kill you in northern Australia. On land, there is the death adder, the tiger snake and the redback spider; in the water, you'd be well advised to give wide berth to the salt water crocodile, the stonefish, the great white shark, and of course, the duck-billed platypus.

The duck-billed platypus?

Consider this: in 1991, a man fishing a river in northern Queensland, Australia happened across a platypus sitting on a log. Thinking it was injured, he picked it up. For his trouble, he spent the next six days in a nearby hospital, suffering from two puncture

wounds in his right hand that resulted in "immediate, sustained, and devastating" pain, against which the usual analgesic drugs were almost completely useless. His hand "remained painful, swollen and with little movement for three weeks. Significant functional impairment . . . persisted for three months".

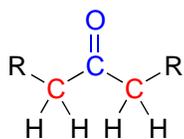
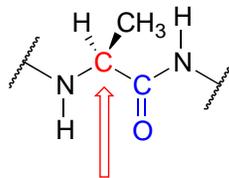
Meanwhile, on the other side of the planet, deep in the rain forests that straddle the border between eastern Peru and Brazil, a young man of the Matses tribe prepares himself to receive the 'hunting magic'. He holds the end of a short wooden stick in a fire for a few minutes, then removes it and presses the red-hot end into the skin of his chest, holding it there for long enough for the skin to be burned. Then he scrapes the burned skin away, and rubs into the wound a paste made from saliva mixed with secretions taken from the skin of a giant leaf frog.

An American journalist named Peter Gorman, who reports having had the frog-skin paste administered in the same manner during a visit to a Matses village, describes what happens next:

Instantly my body began to heat up. In seconds I was burning from the inside . . . I began to sweat. My blood began to race. My heart pounded. I became acutely aware of every vein and artery in my body and could feel them opening to allow for the fantastic pulse of my blood. My stomach cramped and I vomited violently. I lost control of my bodily functions. . . (and) fell to the ground. Then, unexpectedly, I found myself growling and moving about on all fours. I felt as though animals were passing through me, trying to express themselves through my body.

After the immediate violent effects pass, the Matses hunter is carried by his friends to a hammock to recover. After sleeping for a day, he awakens to find himself with what his people call the 'hunting magic': a state of heightened awareness, possessed of tremendous energy and an abnormally keen sense of vision, hearing and smell. In the words of Mr. Gorman, "everything about me felt larger than life, and my body felt immensely strong... [I was] beginning to feel quite godlike".

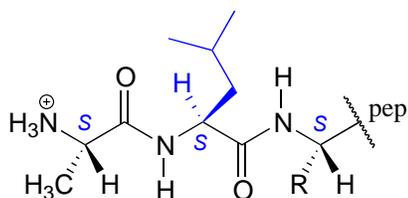
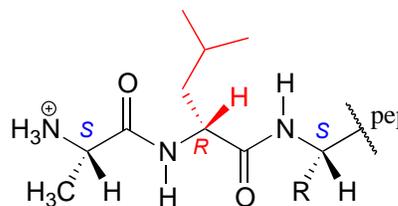
There is a connection between the killer platypus in Australia and the 'hunting magic' in the Amazon, and it has to do with the structure and reactivity of what organic chemists refer to as the **α -carbon**: the carbon atom positioned adjacent to a carbonyl or imine group in an organic molecule:

 α -carbons on a ketonethe α -carbon of an alanine amino acid residue in a protein

It is this chemistry that we are going to be studying for the next two chapters. But first, let's go back to that river in northern Australia and the fisherman who apparently didn't pay enough attention in his high school wildlife biology class.

The platypus, along with a few species of shrews and moles, is an example of a very rare phenomenon in nature: a venomous mammal. The male platypus possesses a pair of sharp spurs on each of his hind legs near the ankle. These spurs are hollow, and connected by a duct to a venom-producing gland in the thigh. The consensus among scientists who study the platypus is that males use their venomous barbs mainly when fighting each other over territory during mating season. Because healthy animals are often found with multiple scars from spur wounds, a platypus who gets spurred during a fight with a rival will not always die, but the experience is unpleasant enough that he will start looking for real estate a healthy distance down the river.

It is not easy to milk the venom from an angry, thrashing platypus, but there are scientists out there who have done it. It turns out that, like snake and spider venom, the venom from a platypus spur consists of a mixture of neuroactive peptides (peptides are very short proteins - less than 50 amino acids long). Recently, a team of biochemists from the Universities of Sydney, Queensland, and Adelaide reported that they were able to isolate from platypus venom two forms of a 39-amino acid peptide. Further analysis using NMR and mass spectrometry revealed that the two forms of the peptide differed in structure only at a single amino acid: the leucine at the #2 position. In one form, the leucine had the L configuration (or *S* if using the *R/S* system), just like the amino acids in virtually all other peptides and proteins found in nature. In the other form, this leucine had the unusual D, or *R* configuration.

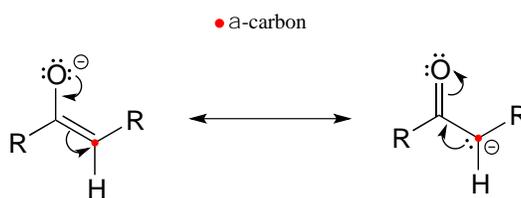
peptide with *L*-Leu at position 2peptide with *D*-Leu at position 2

Peptides or proteins incorporating D-amino acids are not unheard of in nature, but this was the first time that one had been found in a mammal. Interestingly, the venom from certain marine cone snails and spiders - and, yes, the skin of the giant leaf frog in the Amazon rain forest - also contain neuroactive peptides with D-amino acids.

What is the advantage - to a platypus, cone snail, spider, or frog - of making a venomous peptide with *D* stereochemistry on one or more of its amino acids? It all has to do with *generating diversity of shape and function*. These are neuroactive peptides: each one interacts in a very specific way with a specific neural protein, thus exerting a specific neurological effect on the person or animal exposed to the venom. The different spatial arrangement of atoms about the α -carbon of *D*- and *L*-amino acids will cause a peptide with a *D*-leucine at position #2, for example, to fold into a different shape than its counterpart with an *L*-leucine at the same position. Thus, the two peptides may bind differently to one or more proteins in the nervous system, and ultimately may exert different neurological effects - such as intense pain in the case of platypus venom, or the 'hunting magic' effect in the case of the peptide from frog skin. The ability to incorporate *D*-amino acids greatly expands the potential structural and functional diversity of these short peptides.

The two stereoisomeric platypus venom peptides are encoded by the same gene. The peptides are initially synthesized using all *L*-amino acids, and then the leucine at position #2 undergoes a 'post-translational modification': in other words, a specific enzyme binds the all-*L* peptide *after* it has been synthesized on the ribosome and changes the leucine residue to the *D* configuration.

It is this reaction - a stereoisomerization reaction that takes place at the α -carbon of an amino acid - that brings us to the central topic of this chapter and the next: chemistry at the α -carbon. The key concept to recall from what we have learned about acidity and basicity in organic chemistry, and to keep in mind throughout this discussion, is that α -protons (in other words, protons on α -carbons) are weakly acidic. Loss of an α -proton forms an **enolate** - a species in which a negative formal charge is delocalized between a carbon and an oxygen. The 'enolate' term will be very important in the next two chapters, because most of the reactions we see will go through an enolate intermediate.



an enolate

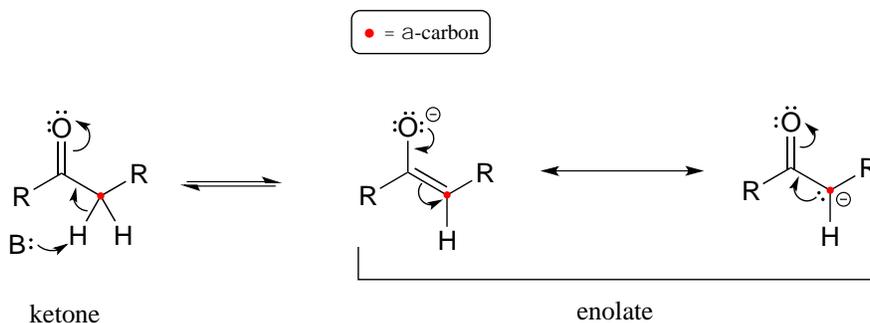
In this chapter, we will first see several examples of isomerization reactions, in which an enzyme acts at the α -carbon of a substrate to catalyze the interconversion of two constitutional isomers or stereoisomers. Then, we will be introduced to a reaction type known as the 'aldol addition' and its reverse counterpart, the 'retro-aldol' cleavage reaction. Up to now, we have seen plenty of reactions where bonds were formed and broken between carbon and oxygen, nitrogen, or sulfur. Here, for the first time, we will see how enzymes can catalyze the formation or cleavage - again, at the α position - of

carbon-carbon bonds: in other words, we will learn how an α -carbon can be either a nucleophile or a leaving group in an enzymatic reaction. This has clear importance for an understanding of metabolism in living things: the molecules of life, after all, are built upon a framework of carbon-carbon bonds, and metabolism is the process by which living cells build up and break down complex biomolecules.

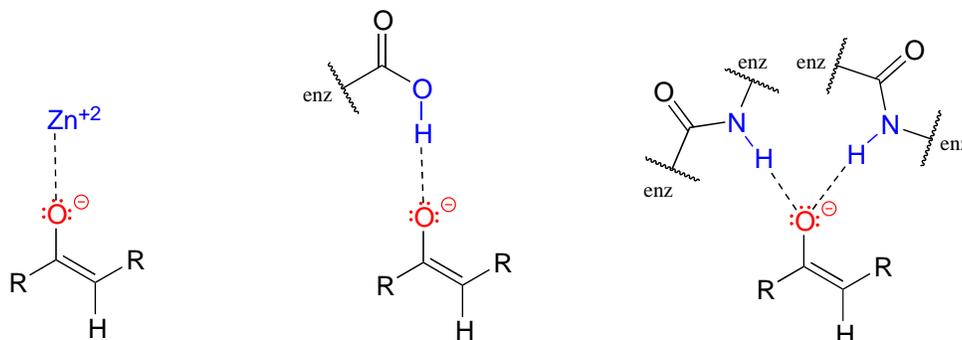
It all starts with the α -carbon - and as both the Australian fisherman and the Amazonian hunter could attest, what happens at the α -carbon can have some rather dramatic consequences.

Section 12.1: Review of acidity at the α -carbon

Let's review what we learned in section 7.6A about the acidity of a proton on an α -carbon and the structure of the relevant conjugate base, the enolate ion. Remember that this acidity can be explained by the fact that the negative charge on the enolate conjugate base is delocalized by resonance to both the α -carbon *and* the carbonyl oxygen.



The α -carbon on the enolate is sp^2 -hybridized with trigonal planar geometry, as are the carbonyl carbon and oxygen atoms (now would be a good time to go back to sections 2.1C, 2.2B, and 2.3B to review, if necessary, the geometry of π -bonding in conjugated systems). The pK_a of a typical α -proton in aqueous solution is approximately 18-20: acidic, but only weakly so. Recall from section 7.8, however, that the *effective* pK_a of a functional group on an enzyme-bound molecule can be altered dramatically by the 'microenvironment' of the active site. In order to lower the pK_a of an α -proton, an enzyme catalyzing a reaction that begins with an α -proton abstraction step must further stabilize the negative charge that develops on the oxygen atom of the (enolate) conjugate base. Different enzymes have evolved different strategies for accomplishing this task: in some cases, a metal cation (often Zn^{+2}) is bound in the active site to provide a stabilizing ion-ion interaction. In other cases, stabilization is provided by a proton-donating group positioned near the oxygen. As a third possibility, the active site architecture sometimes provides one or more stabilizing hydrogen bond donor groups.



In most of the mechanism illustrations in this chapter where an enolate intermediate is depicted, stabilizing metal ions or hydrogen bond interactions will not be explicitly drawn, for the sake of clarity. However, whenever you see an enolate intermediate in an enzyme-catalyzed reaction, you should remember that there are stabilizing interactions in play inside the active site.

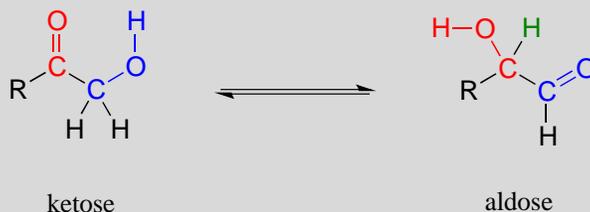
Section 12.2: Isomerization at the α -carbon

Enolate ions, as well as enols and enamines (section 7.6) are the key reactive intermediates in many biochemical isomerization reactions. Isomerizations can involve either the interconversion of constitutional isomers, in which bond connectivity is altered, or of stereoisomers, where the stereochemical configuration is changed. Enzymes that interconvert constitutional isomers are usually called **isomerases**, while those that interconvert the configuration of a chiral carbon are usually referred to as **racemases** or **epimerases**.

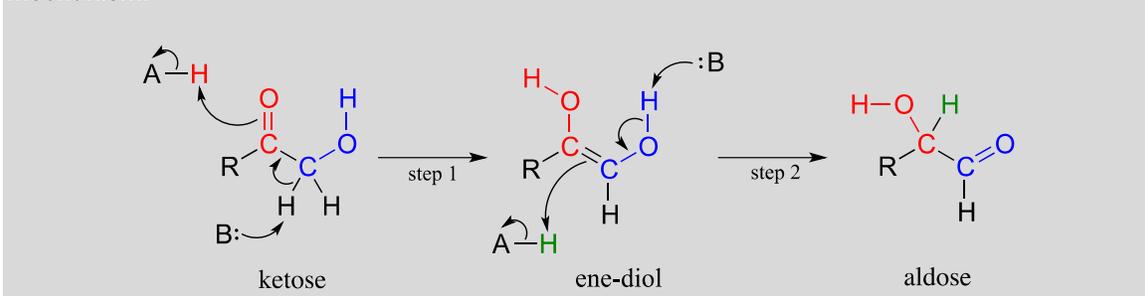
12.2A: Carbonyl isomerization

One very important family of isomerase enzymes catalyzes the shifting of a carbonyl group in sugar molecules, often converting between a ketose and an aldose (recall that the terms ketose and aldose refer to sugar molecules containing ketone and aldehyde groups, respectively).

Carbonyl isomerization:

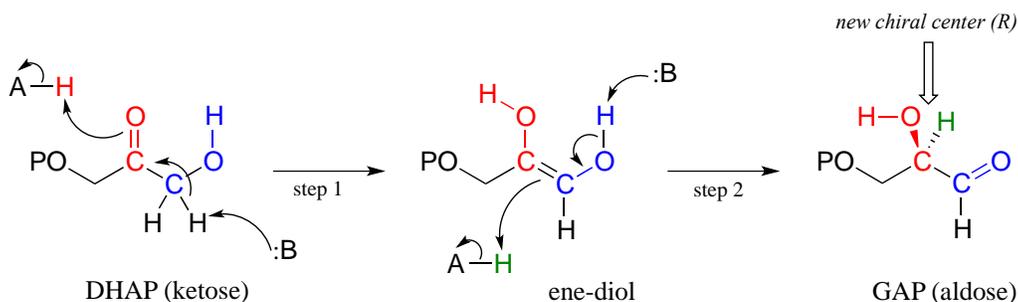


Mechanism:



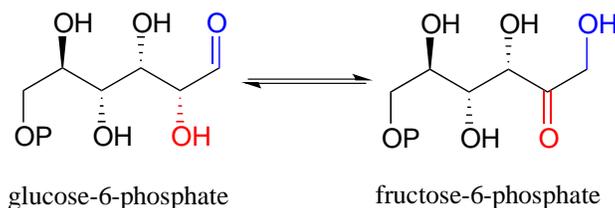
The ketose species is first converted to its enol tautomer in step 1 (actually, this particular intermediate is known as an '**ene-diol**' rather than an enol, because there are hydroxyl groups on both sides of the carbon-carbon double bond). Step 2 leads to the aldose, and is simply another tautomerization step. However, because there is a hydroxyl group on the adjacent (blue) carbon, a carbonyl can form there as well as at the red carbon.

An example is the glycolysis pathway reaction catalyzed by the enzyme triose phosphate isomerase (EC 5.3.1.1). Here, dihydroxyacetone phosphate (DHAP) is reversibly converted to glyceraldehyde phosphate (GAP).



Notice that DHAP is achiral while GAP is chiral, and that a new chiral center is introduced at the middle (red) carbon of GAP. As you should expect, the enzyme is stereoselective: in step 2 a proton is delivered to the red carbon, from *behind* the plane of the page, to yield the *R* enantiomer.

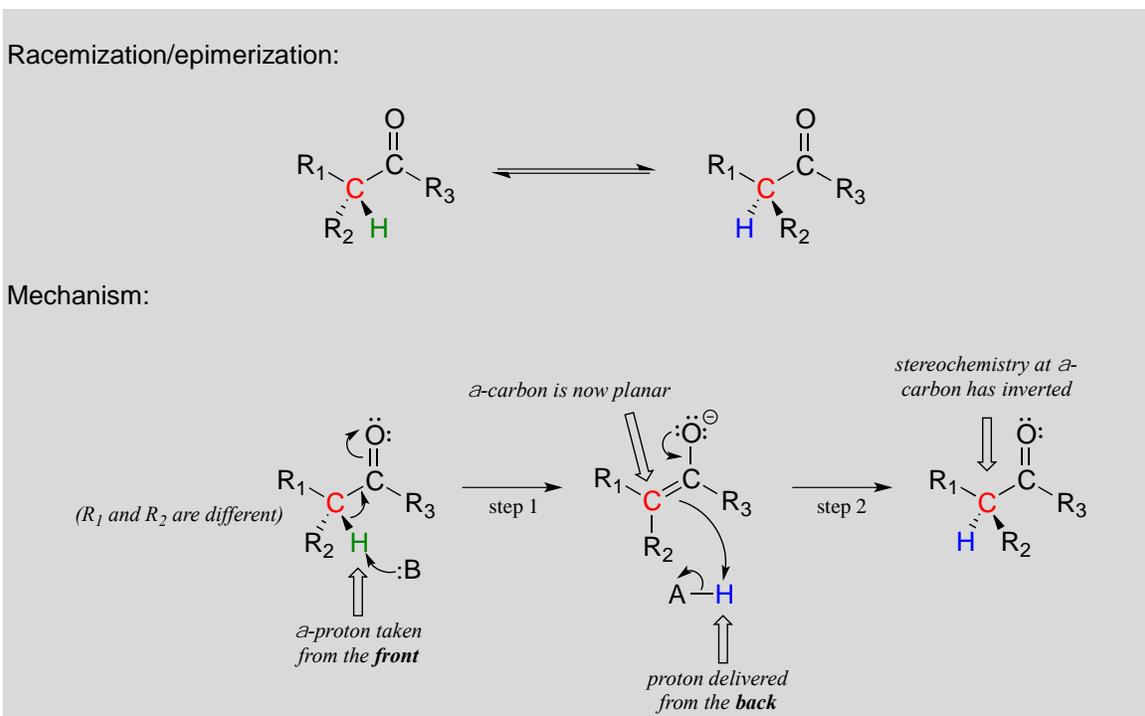
Also in the glycolysis pathway, glucose-6-phosphate (an aldose) and fructose-6-phosphate (a ketose) are interconverted through an ene-diol intermediate (EC 5.3.1.9) by an enzyme that is closely related to triose-phosphate isomerase.



Exercise 12.1: draw the ene-diol intermediate in the phosphoglucose isomerase reaction.

12.2B: Stereoisomerization at the α -carbon

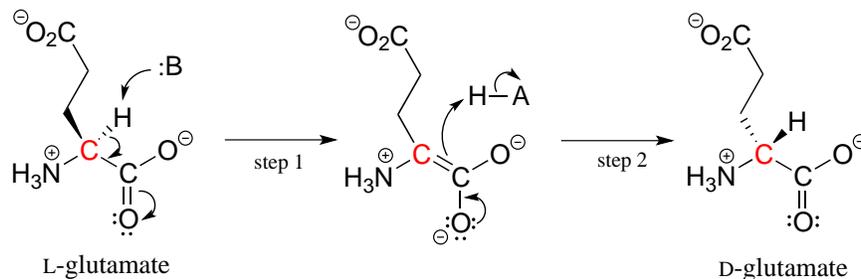
Enolates are a common intermediate in reactions where the stereochemical configuration of a chiral α -carbon is interconverted. These are commonly referred to as **racemization** or **epimerization** reactions, depending on whether the interconverted isomers are enantiomers or epimers (recall that the term 'epimer' refers to a pair of diastereomers that differ by a single chiral center).



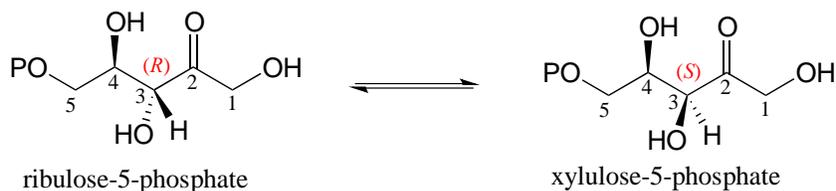
These reactions proceed through a deprotonation-reprotonation mechanism, illustrated above. In step 1, the chiral α -carbon is deprotonated, leading to a planar, achiral enolate. In step 2, a proton is delivered back to the α -carbon, *but from the opposite side from which the proton was taken in step 1*, resulting in the opposite stereochemistry at this carbon. Two acid-base groups, positioned at opposing sides of the enzyme's active site, work in tandem to accomplish this feat.

The proteins and peptides in all known living things are constructed almost exclusively of L-amino acids, but in rare cases scientists have identified peptides which incorporate D-amino acids, which have the opposite stereochemistry at the α -carbon. Amino acid racemase enzymes catalyze the interconversion of L and D amino acids. As you may

recall from the introductory section to this chapter, the venom of the male platypus contains a neurotoxic peptide in which an L-leucine amino acid has been converted by a racemase enzyme to D-leucine. In another example, the cell walls of bacteria are constructed in part of peptides containing D-glutamate, converted from L-glutamate by the enzyme glutamate racemase.

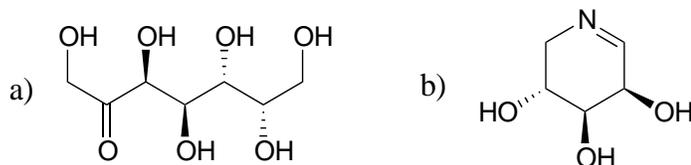


A reaction (EC 5.1.3.1) in sugar metabolism involves the interconversion of the epimers ribulose-5-phosphate and xylulose-5-phosphate. The enzyme that catalyzes this reaction is called an 'epimerase'.

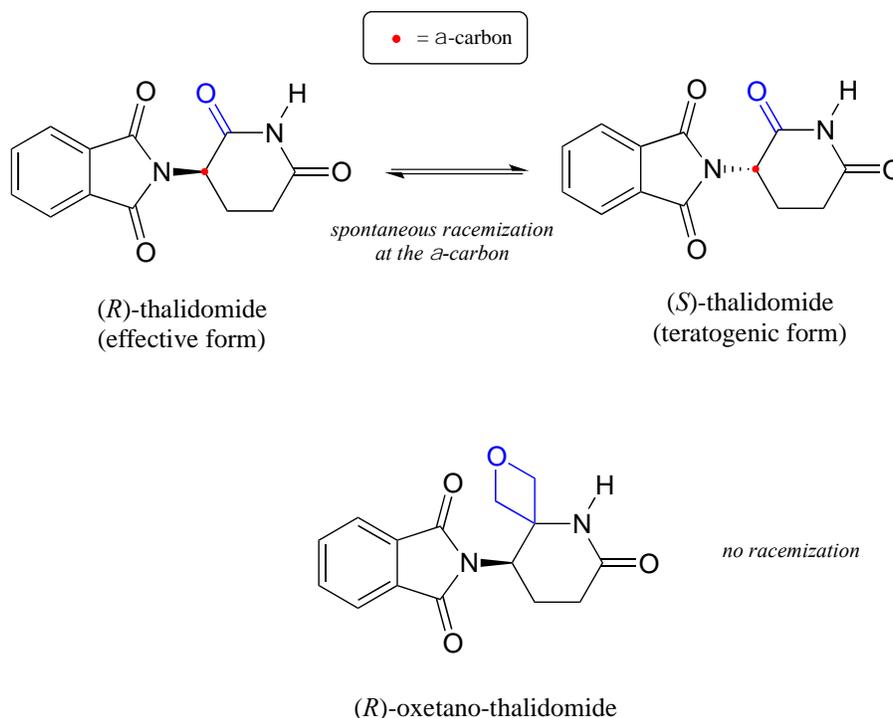


Exercise 12.2: Draw a reasonable mechanism for the ribulose-5-phosphate epimerization reaction above. Your mechanism should show an enolate intermediate and specify stereochemistry throughout.

Exercise 12.3: Predict the products of epimerization reactions starting with each of the substrates shown. Hint - carbons next to imine groups can also be considered α -carbons!



Recall from chapter 3 that a major issue with the drug thalidomide is the fact that the *R* enantiomer, which is an effective sedative, rapidly isomerizes in the body to the teratogenic (mutation-causing) *S* enantiomer. Note that the chiral center in thalidomide is an α -carbon!

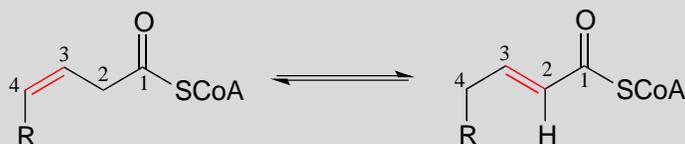


Recently, chemists reported the synthesis of a thalidomide derivative in which the carbonyl group is replaced by an 'oxetane' ring, with the aim of making an isotopically stable form of the drug (because the carbonyl group has been removed, racemization is no longer possible - there is no α -carbon!) (*Org. Lett.* **2013**, *15*, 4312.)

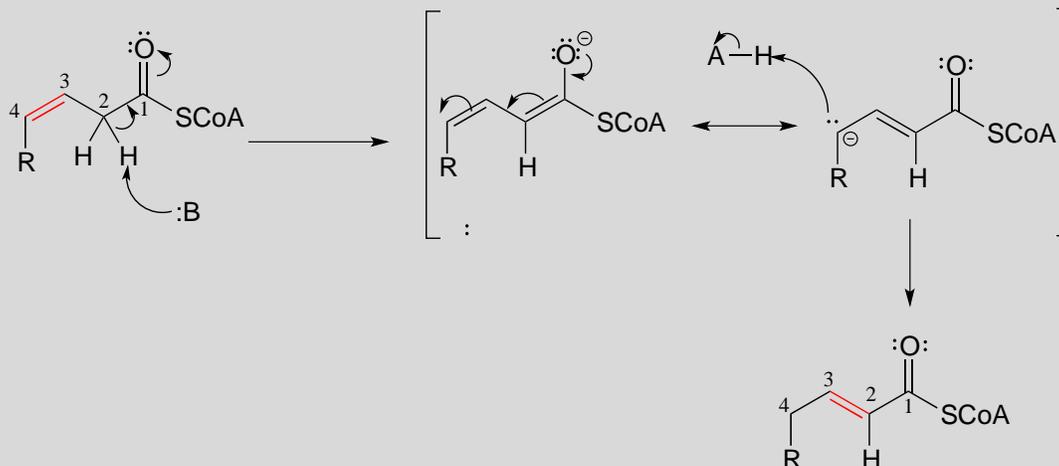
12.2C: Alkene regioisomerization

The position of an alkene group can also be changed through a reaction in which the first step is abstraction of an α -proton and formation of an enolate intermediate. The degradation pathway for unsaturated fatty acids (fatty acids whose hydrocarbon chains contain one or more double bonds) involves the 'shuffling' of the position of a carbon-carbon double bond, from a *cis* bond between carbon #3 and carbon #4 to a *trans* bond between carbon #2 and carbon #3. This is accomplished by the enzyme enoyl CoA isomerase (EC 5.3.3.8).

Alkene isomerization:

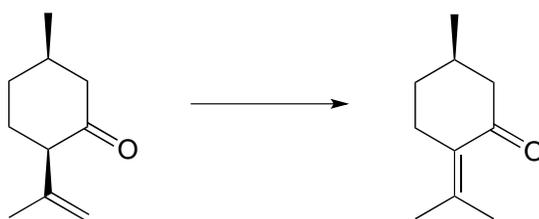


Mechanism:



Exercise 12.4: Consider the structures of the substrate and product of the isomerization reaction above. What two factors contribute to the thermodynamic 'driving force' for the transformation?

Exercise 12.5: The reaction below is part of the biosynthetic pathway for menthol. Suggest a mechanism that includes an enolate intermediate.



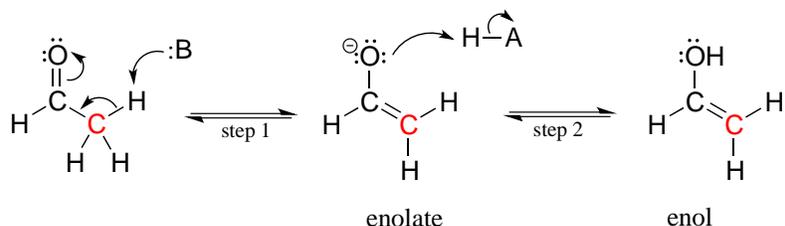
Section 12.3: Aldol addition reactions

We arrive now at one of the most important mechanisms in metabolism: the aldol addition.

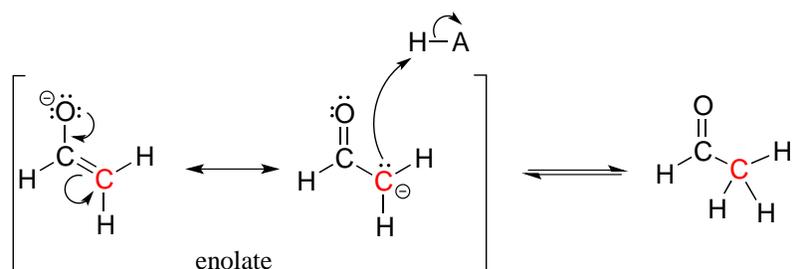
Along with Claisen condensation reactions, which we will study in the next chapter, aldol additions are responsible for most of the carbon-carbon bond forming events that occur in a living cell. Because biomolecules are built upon a framework of carbon-carbon bonds, it is difficult to overstate the importance of aldol addition and Claisen condensation reactions in the chemistry of living things!

12.3A: Overview of the aldol addition reaction

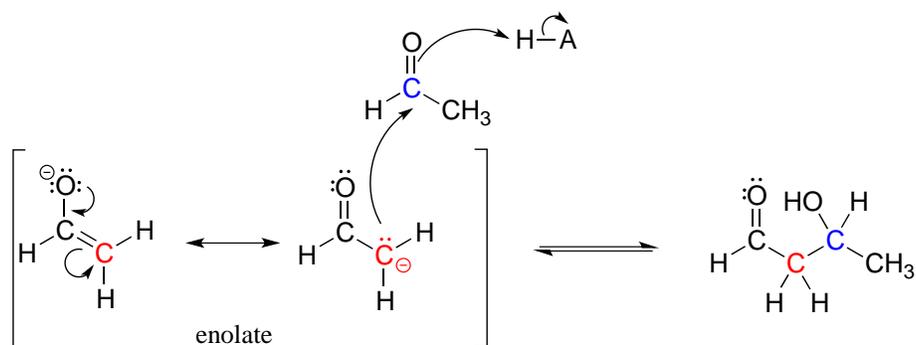
Consider the potential pathways available to a reactive enolate intermediate once the α -proton has been abstracted. We'll use acetaldehyde as a simple example. The oxygen, which bears most of the negative charge, could act as a base, (step 2 below) and the result would be an enol.



Alternatively, the enolate carbon, which bears a degree of negative charge, could act as a base, which is simply the reverse of the initial deprotonation step that formed the enolate in the first place. This of course just takes us right back to the starting aldehyde.

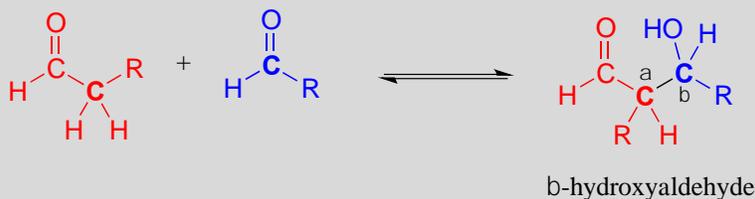


In both of these cases, the electron-poor species attacked by the enolate is an acidic proton. What if the electron-poor species - the electrophile - is not a proton but a carbonyl carbon? In other words, what if the enolate acts not as a base but rather as a *nucleophile* in a carbonyl addition reaction? For example, the enolate of acetaldehyde could attack the carbonyl group of a second acetaldehyde molecule. The result is the formation of a new carbon-carbon bond:

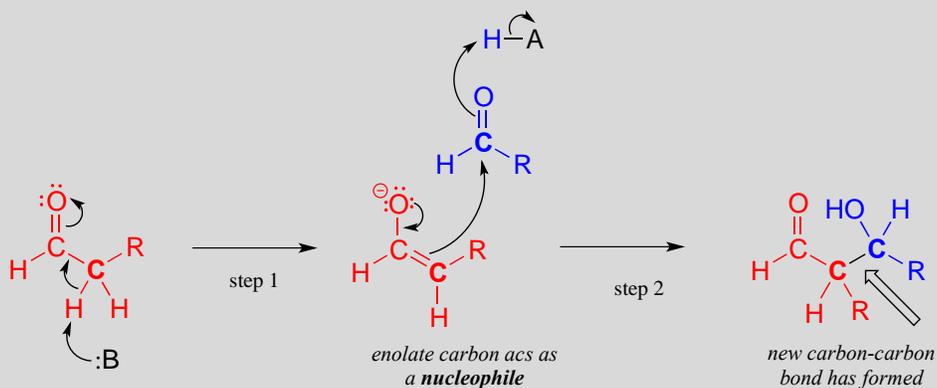


This type of reaction is called an **aldol addition**. *It can be very helpful to think of an aldol addition reaction as simply a nucleophilic carbonyl addition (Chapter 10) reaction with an enolate α -carbon (rather than an alcohol oxygen or amine nitrogen) as the nucleophile.*

An aldol addition reaction:



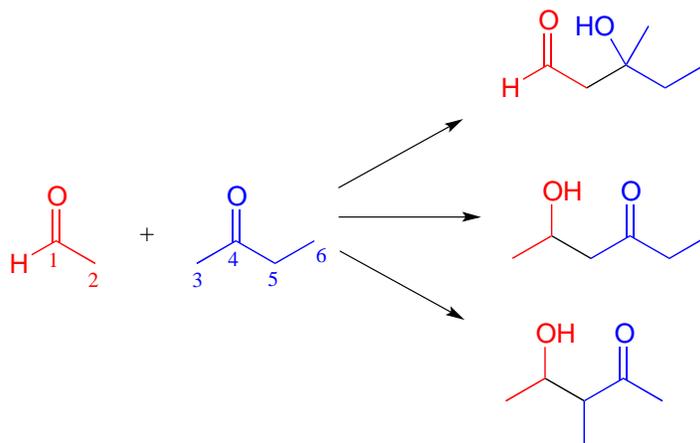
Mechanism:



Historically, the first examples of this mechanism type to be studied involved reactions very similar to what is shown above: an aldehyde reacting with itself. Because the resulting product contained both an **aldehyde** and an **alcohol** functional group, the reaction was referred to as an 'aldol' addition, a terminology that has become standard for reactions of this type, whether or not an aldehyde is involved. More generally, an aldol addition is characterized as a nucleophilic addition to an aldehyde, ketone, or imine electrophile where the nucleophile is the α -carbon in an aldehyde, ketone, imine, ester, or thioester. The enzymes that catalyze aldol reactions are called, not surprisingly, **aldolases**.

Note that the aldol reaction results in a product in which a hydroxide group is *two* carbons away from the carbonyl, in the **β position**. You can think of the β -hydroxy group as a kind of 'signature' for an aldol addition product.

Depending on the starting reactants, *nonenzymatic* aldol reactions can take more than one route to form different products. For example, a reaction between acetaldehyde and 2-butanone could potentially result in three different aldol addition products, depending on which of the three α -carbons (carbons 2, 3, and 5 below) becomes the attacking nucleophile.



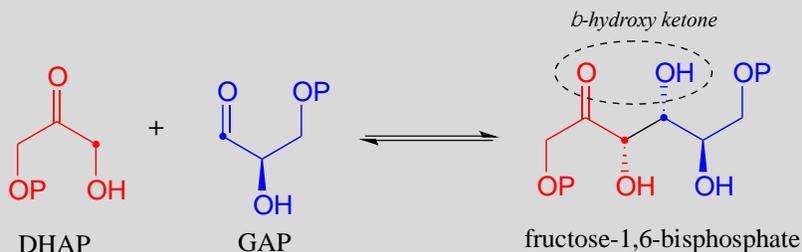
Exercise 12.6: (*Hint:* for each reaction, first identify the nucleophilic and electrophilic carbon atoms on the starting compounds!)

- Fill in the appropriate carbon numbers for each of the three possible aldol addition products shown above.
 - Draw arrows for the carbon-carbon bond forming step that leads to each of the three products.
-

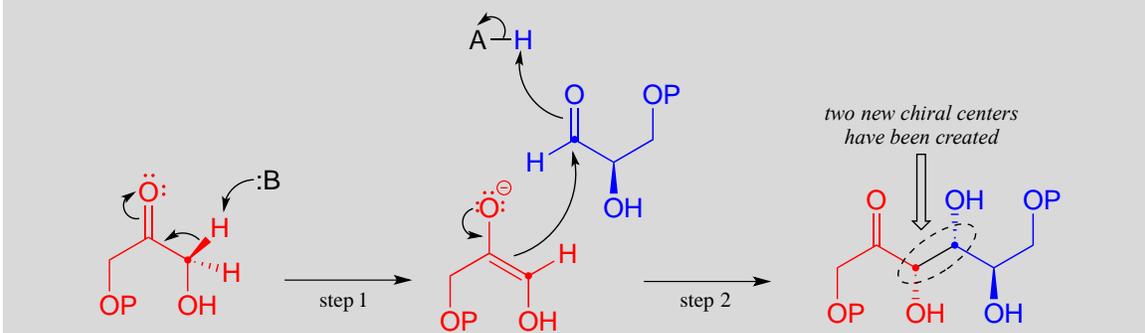
12.3B: Biochemical aldol addition reactions

Fructose 1,6-bisphosphate aldolase (EC 4.1.2.13) is an enzyme that participates in both the glycolytic (sugar catabolism) and gluconeogenesis (sugar synthesis) biochemical pathways. The reaction catalyzed by fructose 1,6-bisphosphate aldolase links two 3-carbon sugars, glyceraldehyde-3-phosphate (GAP, the electrophile in the reaction) and dihydroxyacetone phosphate (DHAP, the nucleophile), forming a 6-carbon product. In the figures below, the nucleophilic and electrophilic carbons are identified with dots.

The fructose 1,6-bisphosphate aldolase reaction



Mechanism:



In step 1 of the reaction, an α -carbon on DHAP is deprotonated, leading to an enolate intermediate. In this and many other aldolase reactions, a zinc cation (Zn^{+2}) is positioned in the enzyme's active site so as to interact closely with - and stabilize - the negatively charged oxygen of the enolate intermediate. This is one important way in which the enzyme lowers the energy barrier to the reaction.

Next, (step 2), the deprotonated α -carbon attacks the carbonyl carbon of GAP in a nucleophilic addition reaction, leading to the fructose 1,6-bisphosphate product.

Notice that two new chiral centers are created in this reaction. This reaction, being enzyme-catalyzed, is highly stereoselective due to the precise position of the two substrates in the active site: only one of the four possible stereoisomeric products is observed. The enzyme also exhibits tight control of regiochemistry: GAP and DHAP could potentially form two other aldol products which are constitutional isomers of fructose 1,6-bisphosphate.

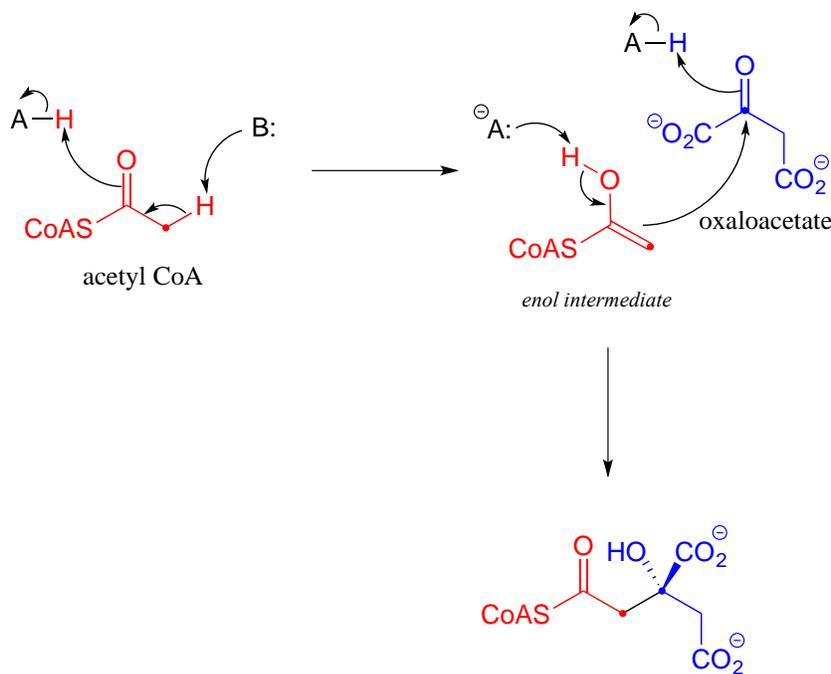
Exercise 12.7:

a) Fill in the blanks with the correct term: (*pro-R*, *pro-S*, *re*, *si*). You may want to review the terminology in section 3.11.

In the fructose 1,6-bisphosphate aldolase reaction, the _____ proton on the α -carbon of DHAP is abstracted, then the _____ face of the resulting enolate α -carbon attacks the _____ face of the aldehyde carbon of GAP.

b) Draw structures of the two other constitutional isomers that could *hypothetically* form in aldol addition reactions between GAP and DHAP. How many stereoisomers exist for these two alternative products?

Along with aldehydes and ketones, esters and thioesters can also act as the nucleophilic partners in aldol reactions. In the first step of the citric acid (Krebs) cycle, acetyl CoA (a thioester nucleophile) adds to oxaloacetate (a ketone electrophile) (EC 2.3.3.8).

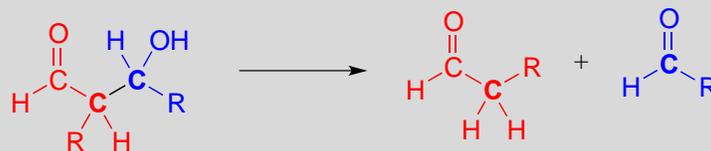


Notice that the nucleophilic intermediate is an *enol*, rather than a zinc-stabilized enolate as was the case with the fructose 1,6-bisphosphate aldolase reaction. An enol intermediate is often observed when the nucleophilic substrate is a thioester rather than a ketone or aldehyde.

12.3C: Going backwards: the retro-aldol cleavage reaction

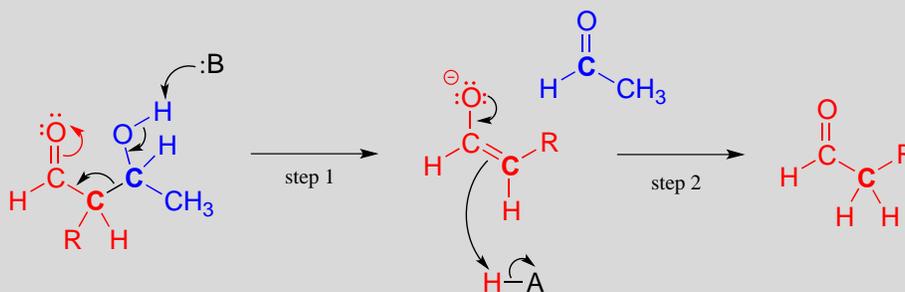
Although aldol reactions play a very important role in the formation of new carbon-carbon bonds in metabolic pathways, it is important to emphasize that they can also be reversible: in most cases, the energy level of starting compounds and products are very close. This means that, depending on metabolic conditions, aldolases can also catalyze **retro-aldol** reactions: the reverse of aldol reactions, in which carbon-carbon bonds are *broken*.

A retro-aldol cleavage reaction:



β -hydroxycarbonyl

Mechanism:

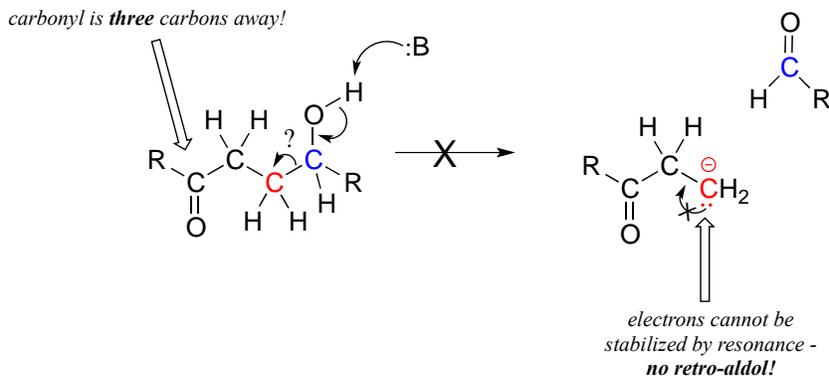


In the retro-aldol cleavage reaction the β -hydroxy group is deprotonated (step 1 above), to form a carbonyl, at the same time pushing off the enolate carbon, which is now a leaving group rather than a nucleophile.

Is an enolate a good enough leaving group for this step to be chemically reasonable? Sure it is: the same stabilizing factors that explain why it can form as an intermediate in the forward direction (resonance delocalization of the negative charge to the oxygen, interaction with a zinc cation) also explain why it is a relatively weak base, and therefore a relatively good leaving group (remember, weak base = good leaving group!). All we need to do to finish the reaction off is reprotonate the enolate (step 2) to yield the starting aldehyde, and we are back where we started.

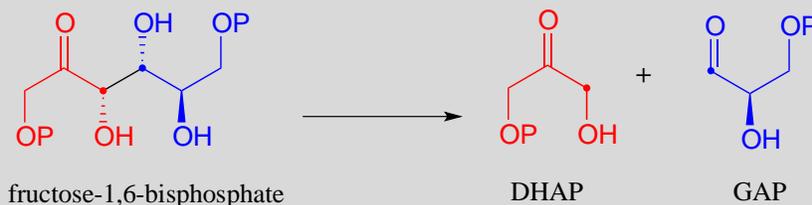
The key thing to keep in mind when looking at a retro-aldol mechanism is that, when the carbon-carbon bond breaks, the electrons must have 'some place to go' where they will be stabilized by resonance. *Most often, the substrate for a retro-aldol reaction is a β -hydroxy aldehyde, ketone, ester, or thioester.*

If the leaving electrons cannot be stabilized, a retro-aldol cleavage step is highly unlikely.

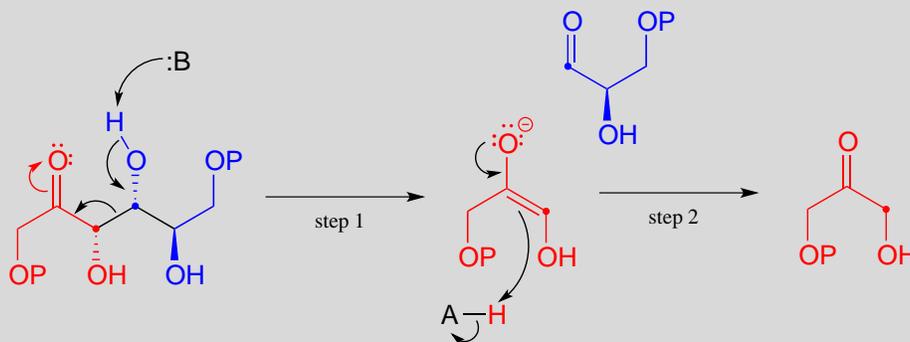


The fructose 1,6-bisphosphate aldolase reaction we saw in the previous section is an excellent example of an enzyme whose metabolic role is to catalyze both the forward and reverse (retro) directions of an aldol reaction. The same enzyme participates both as an aldolase in the sugar-building gluconeogenesis pathway, and as a retro-aldolase in the sugar breaking glycolysis pathway. We have already seen it in action as an aldolase in the gluconeogenesis pathway. Here it is in the glycolytic direction, catalyzing the retro-aldol cleavage of fructose biphosphate into DHAP and GAP:

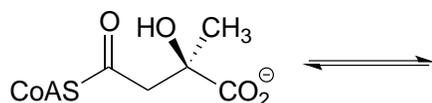
The fructose 1,6-bisphosphate aldolase reaction (retro-aldol direction)



Mechanism:



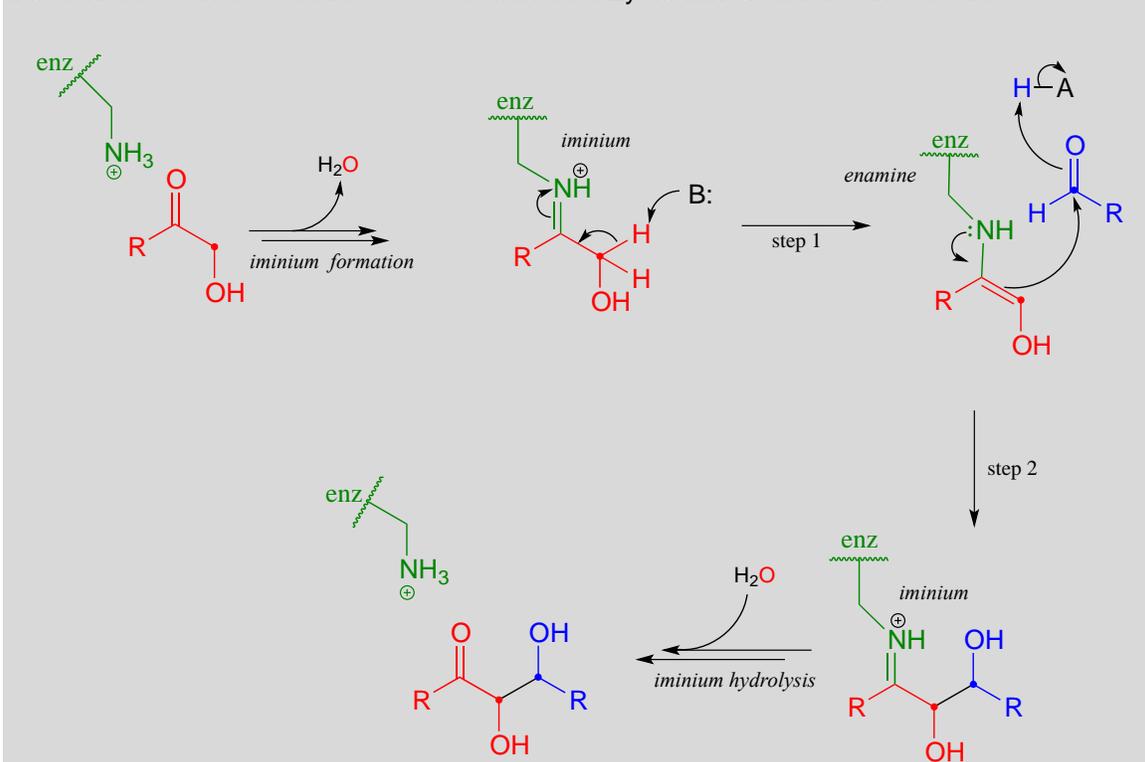
Exercise 12.8: Predict the products of a retro-aldol reaction with the given substrate.



12.3D: Aldol addition reactions with enzyme-linked enamine intermediates

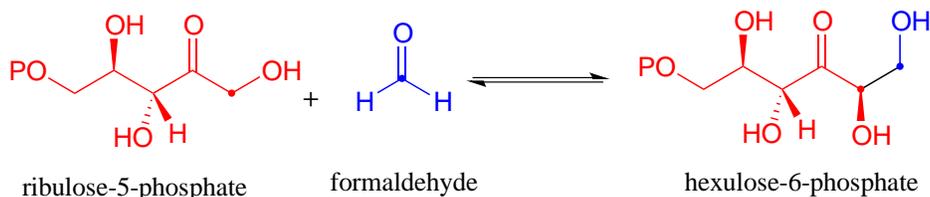
Earlier we looked at the mechanism for the fructose 1,6-bisphosphate aldolase reaction in bacteria. Interestingly, it appears that the enzyme catalyzing the exact same reaction in plants and animals evolved differently: instead of going through a zinc-stabilized enolate intermediate, in plants and animals the key intermediate is an *enamine*. The nucleophilic substrate is first linked to the enzyme through the formation of an iminium with a lysine residue in the enzyme's active site (refer to section 10.5 for the mechanism of iminium formation). *This effectively forms an 'electron sink', in which the positively-charged iminium nitrogen plays the same role as the Zn^{+2} ion in the bacterial enzyme.*

Mechanism for an aldol addition reaction with an enzyme-linked enamine intermediate



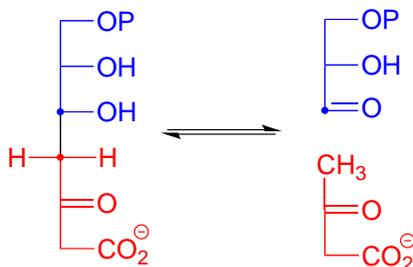
The α -proton, made more acidic by the electron-withdrawing effect of the iminium nitrogen, is then abstracted by an active site base to form an enamine (step 1). In step 2, the α -carbon attacks the carbonyl carbon of an aldehyde, and the new carbon-carbon bond is formed. In order to release the product from the enzyme active site and free the enzyme to catalyze another reaction, the iminium is hydrolyzed back to a ketone group (see section 10.5 to review the imine/iminium hydrolysis mechanism).

There are many more examples of aldol/retroaldol reactions in which the key intermediate is a lysine-linked imine. Many bacteria are able to incorporate formaldehyde, a toxic compound, into carbohydrate metabolism by linking it to ribulose monophosphate. The reaction (EC 4.1.2.43) proceeds through imine and enamine intermediates.



Exercise 12.9: Draw the carbon-carbon bond-forming step for the hexulose-6-phosphate aldolase reaction shown above.

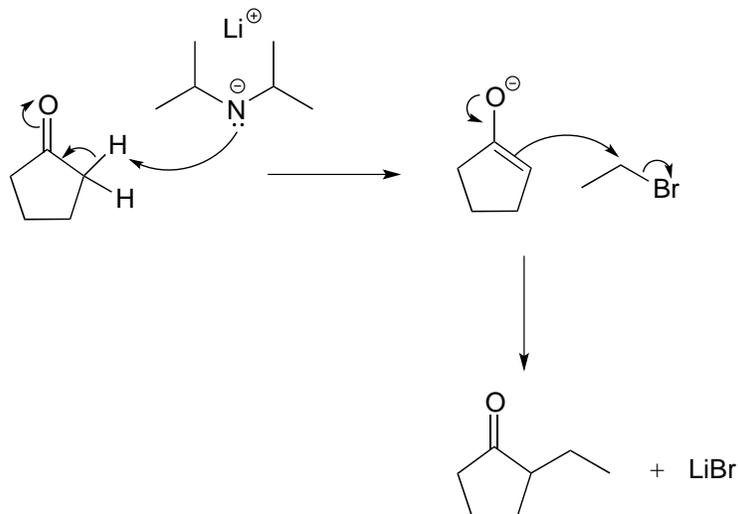
Here is an example of an enamine intermediate retro-aldol reaction from bacterial carbohydrate metabolism (EC 4.1.2.14). Notice that the structures are drawn here in the Fischer projection notation - it is important to practice working with this drawing convention, as biologists and biochemists use it extensively to illustrate carbohydrate chemistry.



Exercise 12.10: Draw the carbon-carbon bond breaking step in the reaction above. Use the Fischer projection notation.

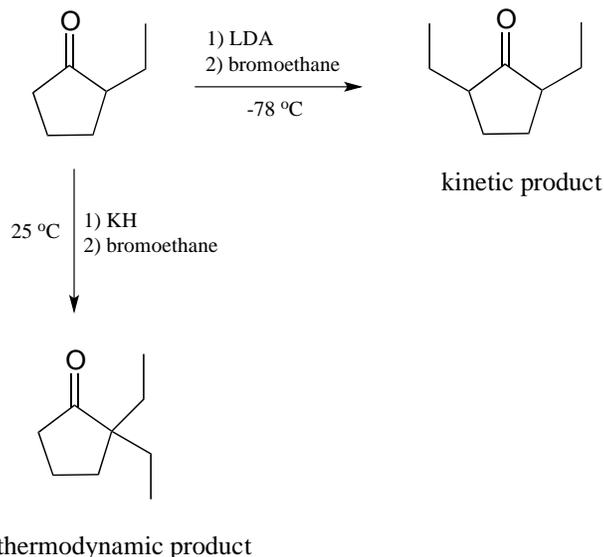
Section 4: α -carbon reactions in the synthesis lab - kinetic vs. thermodynamic enolates

While aldol addition reactions are widespread in biochemical pathways as a way of forming carbon-carbon bonds, synthetic organic chemists working the lab also make use of aldol-like reactions for the same purpose. Consider this reaction:



Here, cyclopentanone is deprotonated at an α -carbon by lithium diisopropylamide (LDA), a very strong base commonly used in the synthesis lab. Addition to the reaction mixture of an electrophile in the form of a primary alkyl bromide results in formation of a new carbon-carbon bond. Notice that this is a kind of ' $\text{S}_{\text{N}}2$ variation' on the aldol addition reactions we saw above, because the enolate nucleophile is attacking in $\text{S}_{\text{N}}2$ fashion rather than in a carbonyl addition fashion.

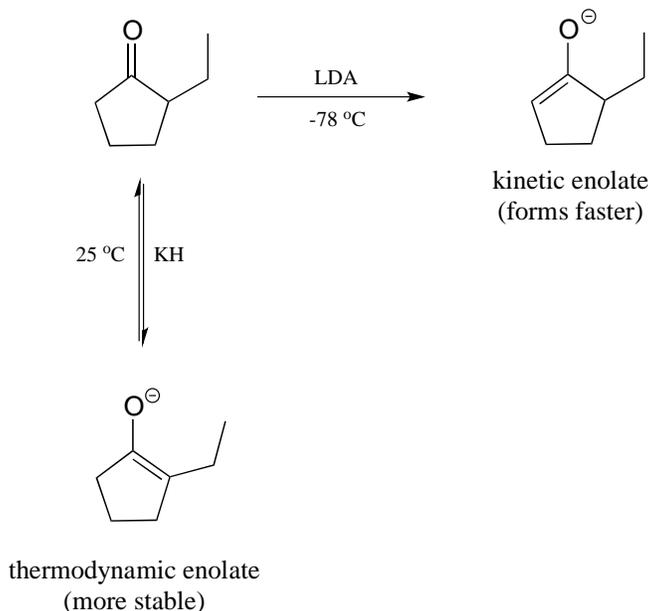
What would happen, though, if we started with 2-ethylcyclopentanone? Because the starting ketone is no longer symmetrical, we could hypothetically obtain two different products:



It turns out that we can control which product we get by selecting the base used in the reaction, and the reaction temperature. If we use LDA and immerse the reaction flask in a dry ice-acetone bath ($-78\text{ }^\circ\text{C}$), we get mainly 2,5-diethyl cyclopentanone. If we use

potassium hydride (KH) and run the reaction at room temperature, we get mainly 2,2-diethylcyclopentanone.

LDA is a very *hindered* base: the basic nitrogen atom is surrounded by two bulky isopropyl groups, and thus it is more difficult for it to come into contact with an α -proton. The α -protons on the *less substituted* side of 2-ethylcyclopentanone are less hindered and more accessible to the base. In addition, the cold reaction temperature means that the deprotonation step is *irreversible*: the system does not have enough energy to overcome the energy barrier for the reverse (reprotonation) reaction. The less substituted enolate forms faster, and once it forms it goes on to attack the bromoethane rather than reversing back to the ketone form. Because it is the *rate* of enolate formation that determines the major product under these conditions, we say that this reaction is under **kinetic control**, and the less substituted enolate intermediate is called the **kinetic enolate**.



If, on the other hand, we use KH as a base, hindrance is no longer an issue because the base is a hydride ion. We run this reaction at room temperature, so the system has enough energy to overcome the energy barrier for re-protonation, and enolate formation is reversible. The enolate in most abundance at equilibrium is therefore not the one that forms fastest, but the one that is *more stable*. The *more substituted* enolate is more stable (recall that alkenes are more stable when they are more substituted - the same idea applies here). The more substituted enolate leads to the 2,2-diethyl cyclopentanone product. Because it is the *stability* of the enolate intermediate that determines the major product under these conditions, we say that this reaction is under **thermodynamic control**, and the more substituted enolate intermediate is the **thermodynamic enolate**.

Key learning objectives for this chapter

Before moving on to the next chapter, you should:

Understand what is meant by ' α and β positions' relative to a carbonyl group.

Understand how an enzyme can increase the acidity of an α -proton through the active site microenvironment

Understand the 3D bonding arrangement of an enolate ion

Be able to recognize and draw reasonable mechanisms for the following reaction types:

tautomerizations: keto-enol, imine-enamine

racemization/epimerization

carbonyl isomerization (changing position of a carbonyl group)

alkene isomerization (changing position of an alkene relative to carbonyl)

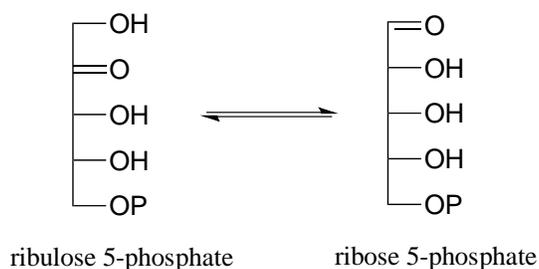
aldol addition, retro-aldol cleavage (both enolate intermediate and enamine intermediate mechanisms)

Be able to draw a mechanism for a laboratory alkylation reaction at the α -carbon of a ketone or aldehyde. Understand the difference between kinetic and thermodynamic control of this reaction type, and be able to predict the regiochemical outcome of the reaction based on reaction conditions.

Problems

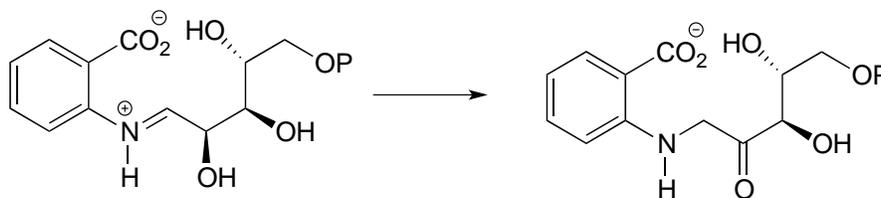
P12.1: The enzyme ribulose-5-phosphate isomerase (EC 5.3.1.6), which is active in both the Calvin cycle and the pentose phosphate pathway, catalyzes an aldehyde-to-ketone isomerization between two five-carbon sugars.

a) Draw a mechanism for this step.



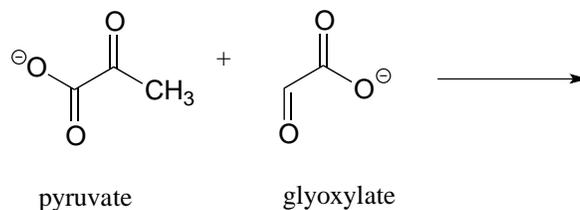
b) What $^1\text{H-NMR}$ signal would most clearly differentiate the aldose from the ketose in this reaction?

P12.2: Provide a likely mechanism for the reaction below, from tryptophan biosynthesis (EC 5.3.1.24) *Hint:* this is mechanistically very similar to a carbonyl isomerization reaction.



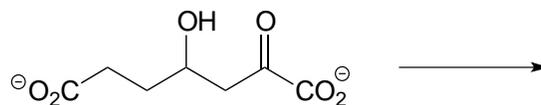
P12.3:

a) Draw the product of an aldol addition reaction between pyruvate and glyoxylate (EC 4.1.3.16):

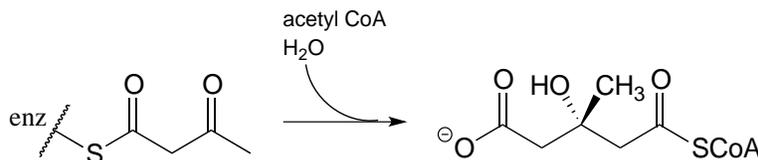


b) Draw the product of an aldol addition reaction between two molecules of pyruvate (EC 4.1.3.17).

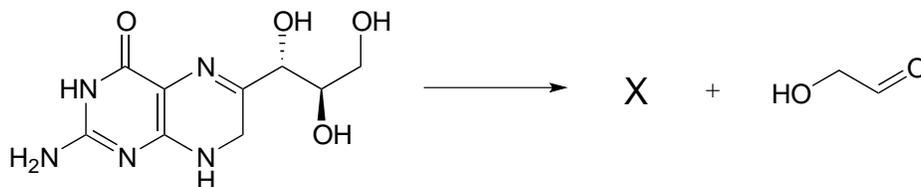
c) The molecule below undergoes a retroaldol cleavage reaction in *E. coli* (*J. Biol. Chem.* **2012**, 287, 36208). Draw the structure of the products.



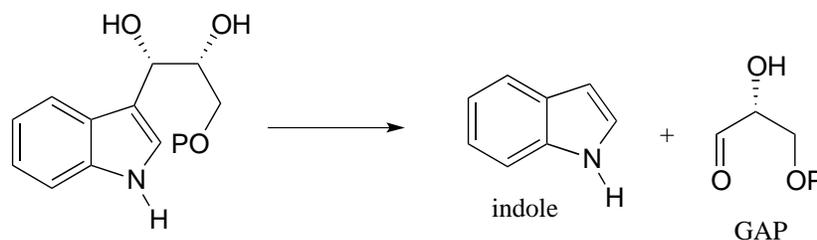
d) Propose a mechanism for this early reaction in the biosynthesis of isoprenoids (EC 2.3.3.10). *Hint*: this is an aldol reaction, followed by thioester hydrolysis.



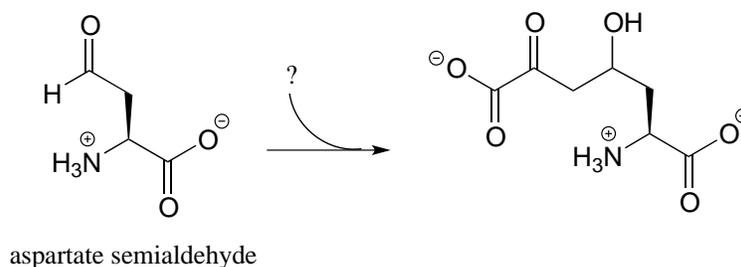
e) The carbon-carbon bond cleaving reaction below was reported to take place in many species of bacteria. Predict the structure of product X, and draw a mechanism for the reaction. Assume that an imine linkage to an enzymatic lysine residue does *not* play a part in the mechanism. (*J. Bacteriol.* **2009**, 191, 4158).



P12.4: Below is a step in the biosynthesis of tryptophan. Draw a likely mechanism. *Hint*: you will need to show an enamine to imine tautomerization step first, then the carbon-carbon bond breaking step will become possible.

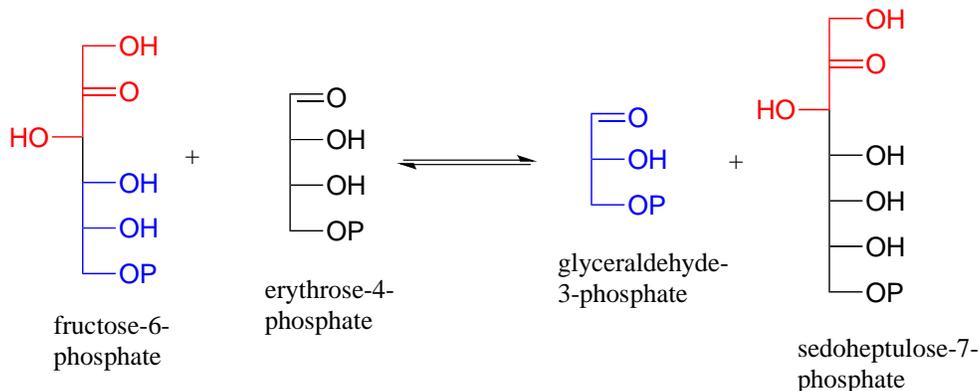


P12.5: The following step in the biosynthesis of lysine makes a connection between aspartate semialdehyde and a common metabolic intermediate. Identify the intermediate, and propose a mechanism for the reaction.

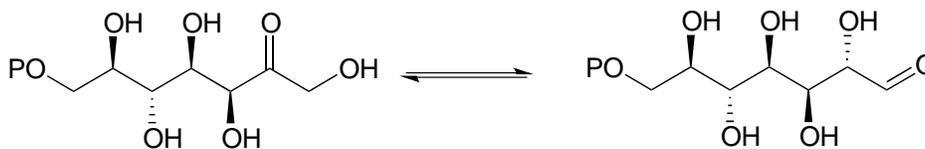


P12.6: Sugar-interconverting transaldol reactions play an important role in sugar metabolism. In a transaldolase reaction, a ketose (e.g. fructose-6-phosphate) first breaks apart in a retro-aldol step to release an aldose (e.g. glyceraldehyde-3-phosphate) from the active site. Then, in a forward aldol step, a second aldose (e.g. erythrose-4-phosphate) enters the active site and connects to what remains from the original ketose (the red part in the figure below) to form a new ketose (e.g. sedoheptulose-7-phosphate). Transaldolase enzymes generally have a lysine in the active site that is covalently bound to the substrate throughout the reaction cycle.

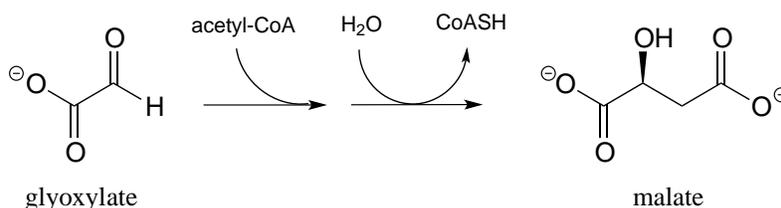
Draw curved-arrow diagrams showing a) the carbon-carbon bond breaking step of the reaction cycle, and b) the carbon-carbon bond forming step.



12.7: Scientists are investigating the enzymatic reaction below, which is part of the biosynthesis of the outer membrane of gram-negative bacteria, as a potential target for new antibiotic drugs. Draw a likely mechanism for the reaction. (*J. Biol. Chem.* **2008**, 283, 2835).



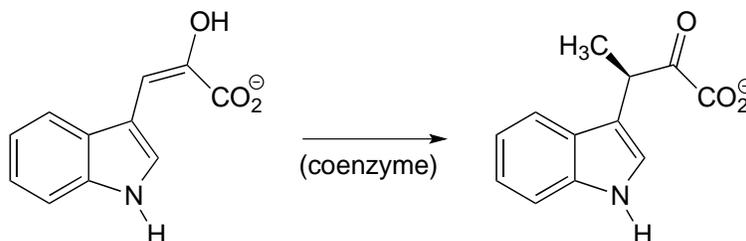
12.8: The reaction below, catalyzed by the enzyme malate synthase, is part of the glyoxylate cycle of plants and some bacteria. It is the glyoxylate cycle that allows these organisms to convert acetyl CoA, derived from the metabolism of oils, into glucose.



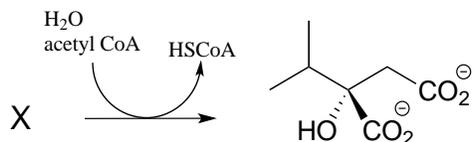
a) Propose a mechanism.

b) Predict the signals you would expect to see in a $^1\text{H-NMR}$ spectrum of malate.

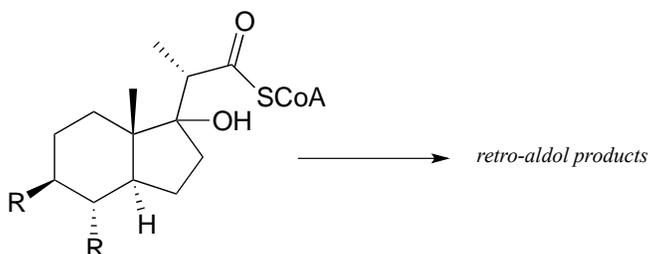
P12.9 The reaction below, from the biosynthetic pathway for the amino acid tryptophan, is dependent upon a coenzyme that we learned about in an earlier chapter. Based on the reaction, identify this coenzyme and propose a mechanism.



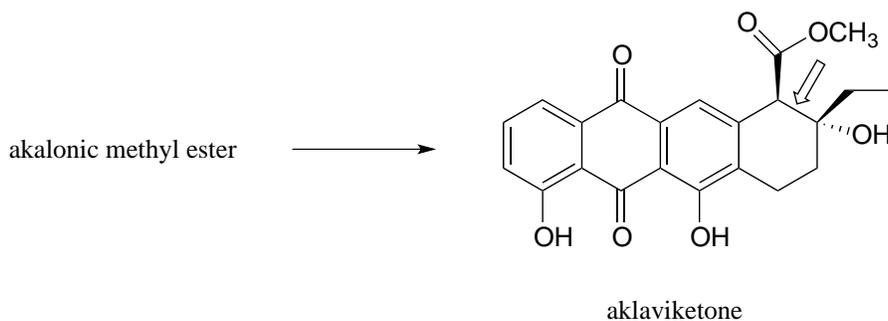
P12.10: In the biosynthesis of leucine, acetyl CoA condenses with another metabolic intermediate 'X' to form 1-isopropylmalate (EC 2.3.3.13). Give the structure for substrate X, and provide a mechanism for the reaction.

**P12.11:**

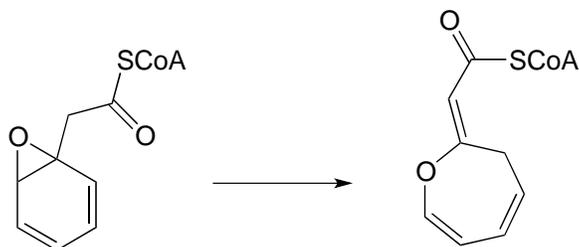
a) *Mycobacterium tuberculosis*, the microbe that causes tuberculosis, derives energy from the metabolism of cholesterol from infected patients. The compound below is predicted to be an intermediate in that metabolic pathway, and to undergo a retro-aldol cleavage reaction. Predict the retro-aldol products and show the mechanism involved. (*Crit. Rev. Biochem. Mol. Biol.* **2014**, 49, 269, fig 5).



b) Polyketides are a structurally diverse class of biomolecules produced by almost all living things. Many drugs are derived from polyketide precursors. The cancer drug doxorubicin (trade name Adriamycin) is derived from a bacterial polyketide called rhodomycinone. Aklaviketone, an intermediate in the biosynthesis of rhodomycinone, is derived in a single enzymatic step from akalonic methyl ester, in a reaction in which the carbon-carbon bond indicated by an arrow is formed. Predict the structure of akalonic methyl ester.

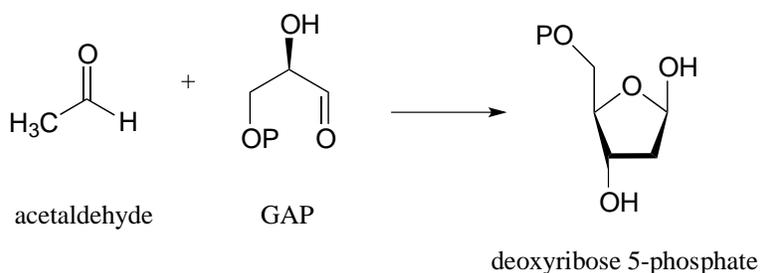


P12.12: The unusual isomerization reaction shown below has been reported recently to occur in some bacteria. Propose a mechanism that begins with formation of an enolate intermediate. (*J. Biol. Chem.* **2012**, 287, 37986).

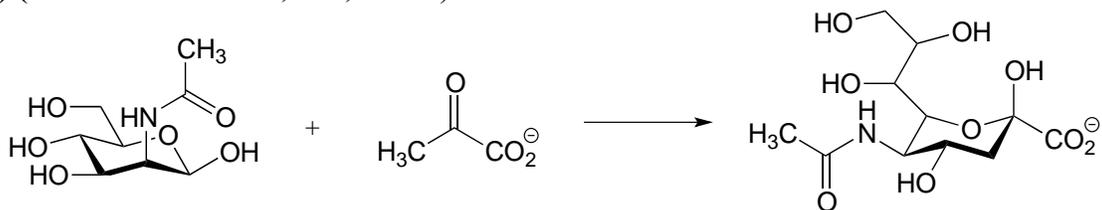


P12.13: The reactions in parts a) and b) below both proceed through lysine-linked enamine intermediates. Show the carbon-carbon bond forming step for each reaction.
Hint: you will want to consider the straight-chain (ie. aldose/ketose) form of the sugars in both cases.

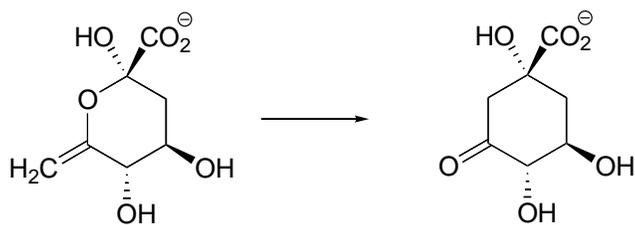
a) (*J. Bacteriol.* **2004**, 186, 4185)



b) (*J. Biol. Chem.* **2011**, 286, 14057)



P12.14: Suggest a mechanism for the following transformation from aromatic amino acid biosynthesis (EC 4.2.3.4). Hint – only two mechanistic steps are required.

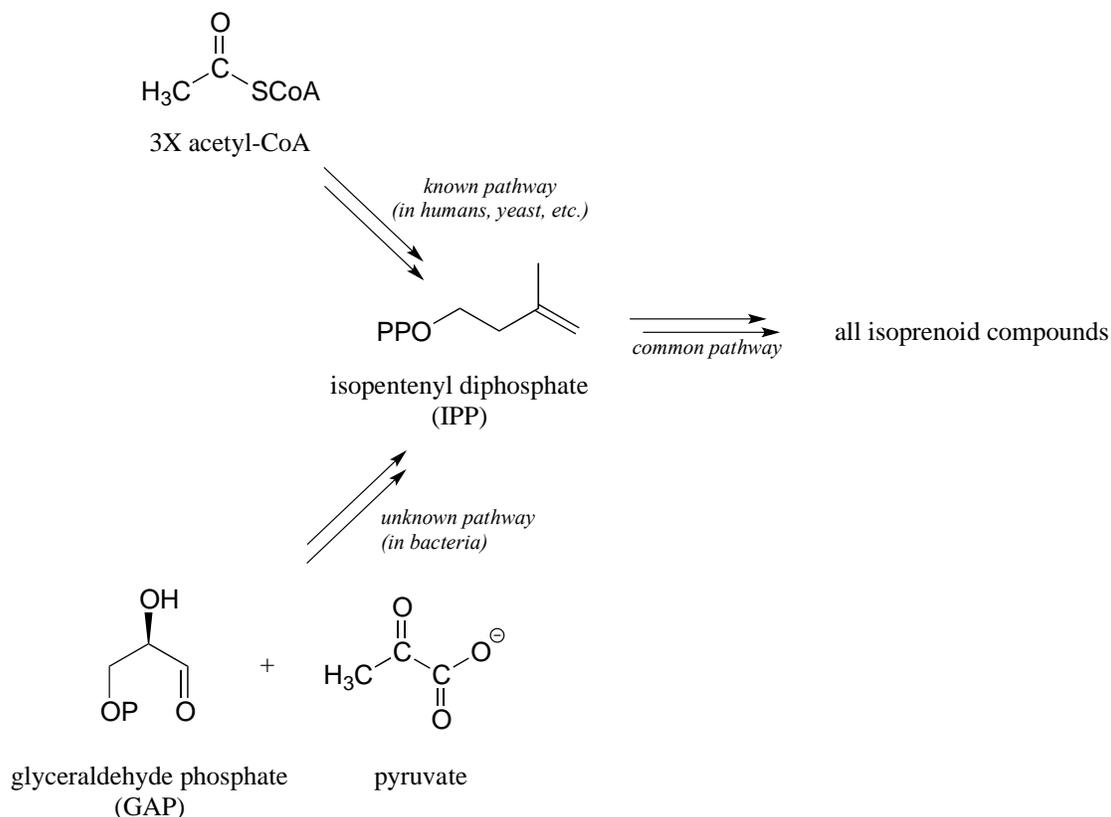


Interchapter

Predicting metabolic pathways

Imagine that you are a biological chemist doing research on bacterial metabolism. You and your colleagues isolate an interesting biomolecule from a bacterial culture, then use mass spectrometry, NMR, and other analytical techniques to determine its structure. Using your 'toolbox' of known organic reaction types - nucleophilic substitution, phosphorylation, aldol additions, and so forth - can you figure out a chemically reasonable pathway by which your compound might be enzymatically synthesized from simple metabolic precursors? In other words, can you fill in the missing biochemical steps (or at least some of them) to come up with a potential new metabolic pathway, which can then be used as a hypothesis for future experimental work to try to find and study the actual enzymes involved?

An actual example approximating this scenario is shown below. A complete biosynthetic pathway for isopentenyl diphosphate (IPP), the building block molecule for all isoprenoid compounds (section 1.3A), has been known since the 1960's. This pathway, which begins with acetyl-CoA, was shown to be active in yeast, plants, and many other species including humans. However, researchers in the late 1980s uncovered evidence indicating that the known pathway is *not* present in bacteria, although they clearly use IPP as a building block molecule just as other forms of life do.



Over the next several years, the researchers conducted a number of experiments in which bacteria were grown on a medium containing glucose 'labeled' with the ^{13}C isotope. With the results from these experiments, combined with their knowledge of common biological organic reaction types, the researchers were able to correctly predict that the bacterial pathway starts with *two* precursor molecules (pyruvate and glyceraldehyde phosphate instead of acetyl CoA) and they also correctly predicted the first two enzymatic steps of the newly discovered bacterial pathway. This accomplishment eventually led to elucidation of every step in the pathway, and isolation of the enzymes catalyzing them. (*Biochem J.* **1993**, 295, 517; *J. Am. Chem. Soc.* **1996**, 118, 2564; *Lipids* **2008**, 43, 1095)

Why weren't they able to predict the whole pathway? It turns out that several of the later steps were somewhat unusual, unfamiliar reaction types - but discovery of these reactions hinged upon the correct prediction of the more familiar first two steps.

Multi-step transformation problems of this type offer an unparalleled opportunity to use our knowledge of biological organic chemistry combined with creative reasoning to solve challenging, relevant scientific puzzles. At this point in your organic chemistry career, you have not yet accumulated quite enough tools in your reaction toolbox to tackle most real-life biochemical pathway problems such as the one addressed above - but by the time we finish with oxidation and reduction chemistry in chapter 15, you will be able to

recognize most of the reaction types that you will encounter in real metabolism, and will be challenged to predict some real pathways in the end-of-chapter problems.

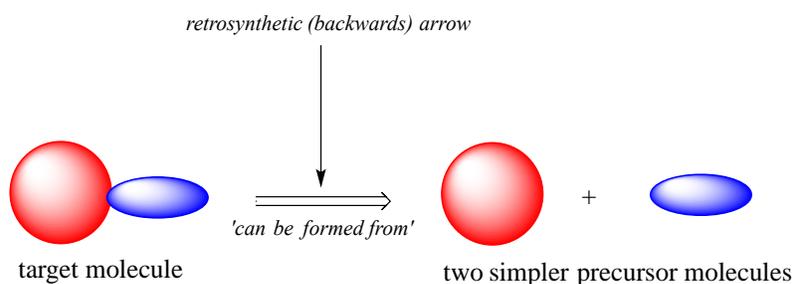
You do, however, have *right now* enough of a bioorganic repertoire to begin to learn how multi-step pathway problems can be approached, using for practice some generalized, hypothetical examples in which the reaction types involved are limited to those with which you are already familiar.

Imagine that you want to figure out how an old-fashioned mechanical clock is put together. One way to do this is to start with a working clock, and take it apart piece-by-piece. Alternatively, one could start with all of the disassembled pieces, plus a lot of other small parts from different clocks, and try to figure out how to put together the specific clock you are interested in. Which approach is easier? The answer is intuitively obvious - it's usually easier to take things apart than to put them back together.

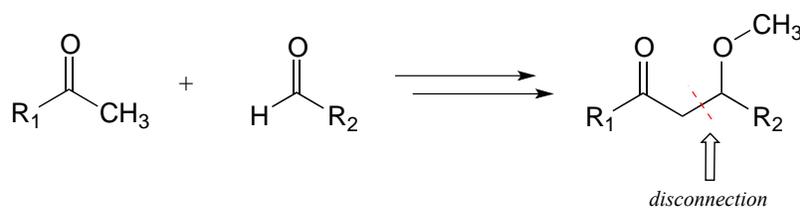
The same holds true for molecules. If we want to figure out the biosynthetic pathway by which a large, complex biomolecule might be made in a cell, it makes sense to start with the finished product and then mentally work backwards, taking it apart step-by-step using known, familiar reactions, until we get to simpler precursor molecules. Starting with a large collection of potential precursor molecules and trying to put the right ones together to make the target product would be a formidable task.

Retrosynthetic analysis - the concept of mentally dismantling a molecule step by step all the way back to smaller, simpler precursors using known reactions - is a powerful and widely-used intellectual tool first developed by synthetic organic chemists. The approach has also been adapted for use by biological chemists in efforts to predict pathways by which known biomolecules could be synthesized (or degraded) in living things.

In retrosynthesis, we think about a series of reactions in reverse. A backwards (retro) chemical step is symbolized by a 'thick' arrow, commonly referred to as a **retrosynthetic arrow**, and visually conveys the phrase '*can be formed from*'.

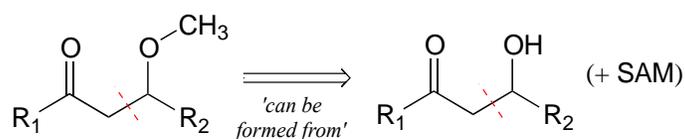


Consider a simple, hypothetical example: starting with the target molecule below, can we come up with a chemically reasonable pathway starting from the precursors indicated?

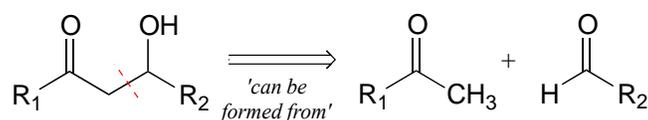


A first step is to identify the relevant **disconnection**: a key bond (usually a carbon-carbon bond) that must be formed to make the target product from smaller precursors. We search our mental 'toolbox' of common biochemical reaction types, and remember that the only way we know of (so far!) to make a new carbon-carbon bond is through an aldol addition reaction, which takes place at an α -carbon. Therefore, we can make a likely disconnection next to the α -carbon in the target molecule.

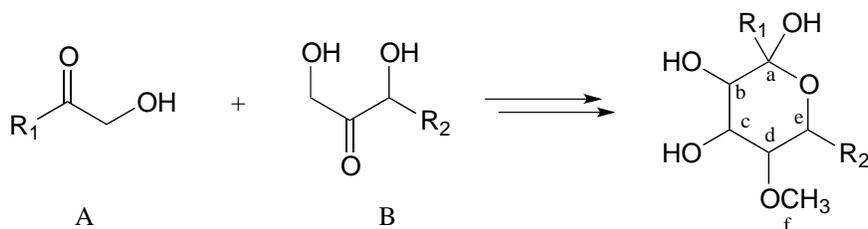
Next, we need to recognize that the aldol addition reaction results in a β -hydroxy ketone. But our target molecule is a β -methoxy ketone! Working backwards, we realize that the β -methoxy group could be formed from a β -hydroxy group by a SAM methylation reaction (section 8.8A). This is our first retrosynthetic (backwards) step.



The second retro step (aldol) accounts for the disconnection we recognized earlier, and leads to the two precursor molecules.

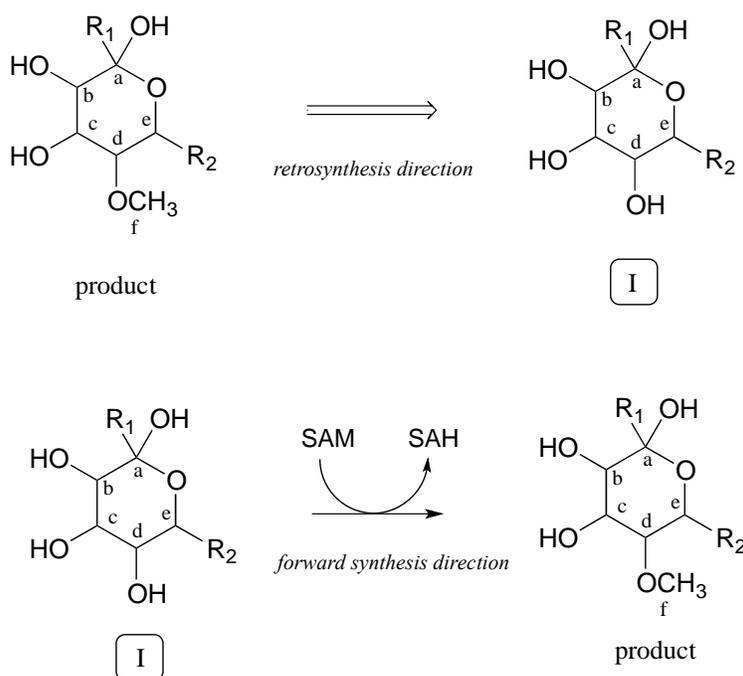


Now, consider the more involved (but still hypothetical) biochemical transformation below:



Often the best thing to do first in this type of problem is to count the carbons in the precursor compounds and product - this allows us to recognize when extra carbons on either side must at some point be accounted for in our solution. In this case, one carbon (labeled 'f') has been *gained* in the form of a methyl ether in the product. This is easy to account for: we know that the coenzyme *S*-adenosyl methionine (SAM - section 8.8A) often serves as the methyl group donor in enzymatic *O*- or *N*-methylation reactions. So, we can propose our first backwards (retro) step: the product as shown could be derived from SAM-dependent methylation of an alcohol group on a proposed intermediate I.

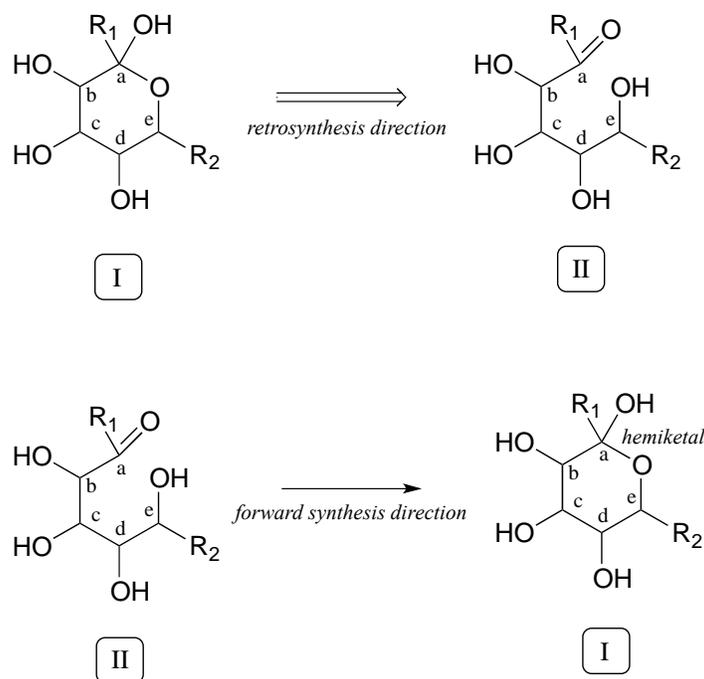
Retrosynthetic step 1:



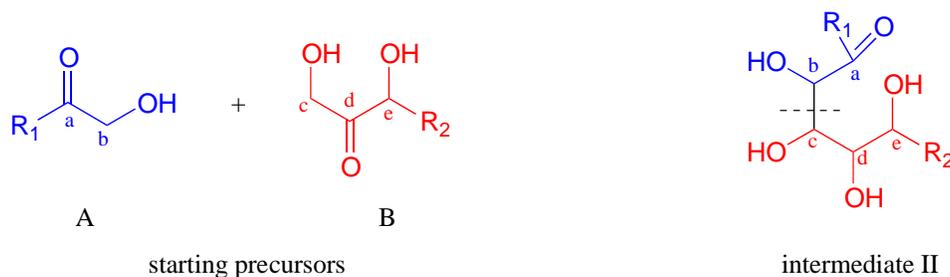
How do we know that the methylation step occurs last? We don't - remember, we are proposing a *potential* pathway, so the best we can do is propose steps that make chemical sense, and which hopefully can be confirmed or invalidated later through actual experimentation. For now, we'll stick with our initial choice to make the methylation step the last one.

Now that we have accounted for the extra carbon, a key thing to recognize regarding the transformation in question is that two linear molecules are combining to form a cyclic product. Thus, *two* connections need to be made between reactants A and B, one to join the two, the other to close the circle. Our primary job in the retro direction, then, is to establish in the product the two points of *disconnection*: in other words, to find the two bonds in the product that need to be taken apart in our retrosynthetic analysis. Look closely at the product: what functional groups do you see? Hopefully, you can identify two alcohol groups, a methyl ether, and (critically) a *cyclic hemiketal*. We've already accounted for the methyl ether. Identifying the cyclic hemiketal is important because it

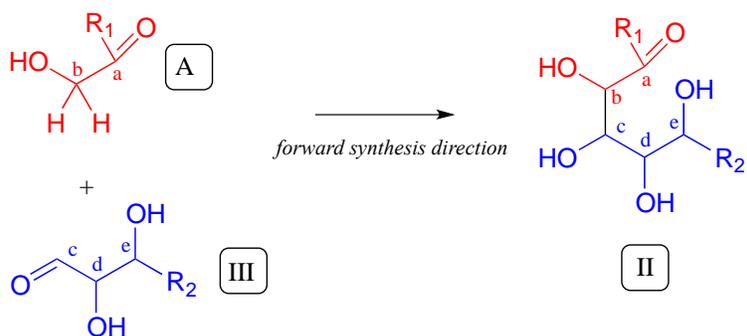
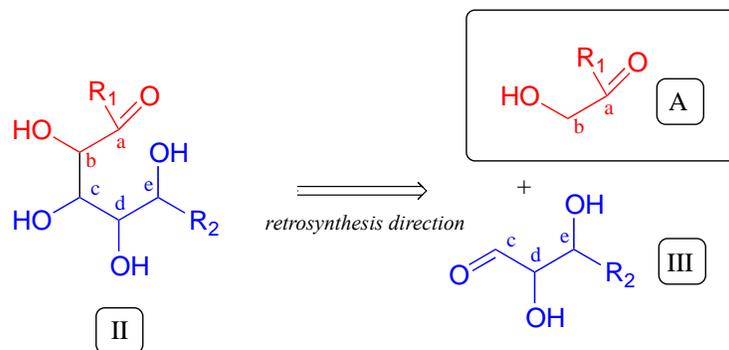
allows us to make our next 'disconnection': we know how a hemiketal forms from a ketone and an alcohol (section 10.2), so we can mentally work backwards and predict the open-chain intermediate II that could cyclize to form our product.



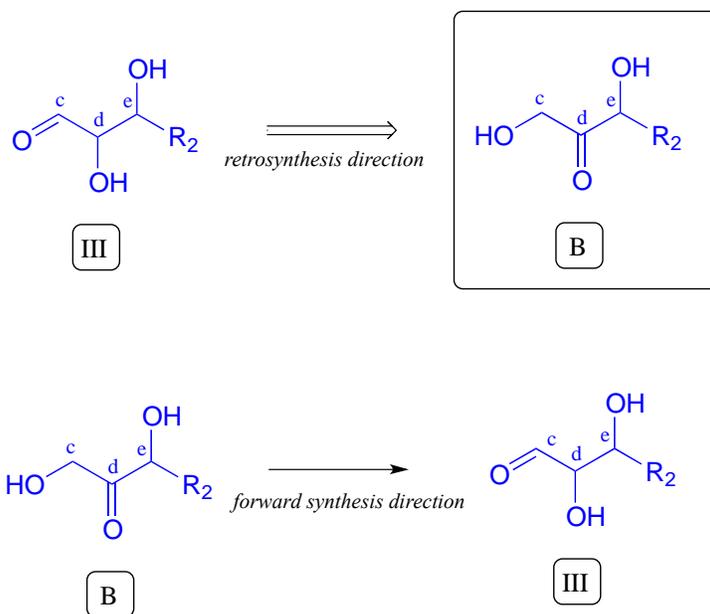
Now, starting with the R_1 group and working along the carbon chain, we can account for carbons a-e on the two precursors.



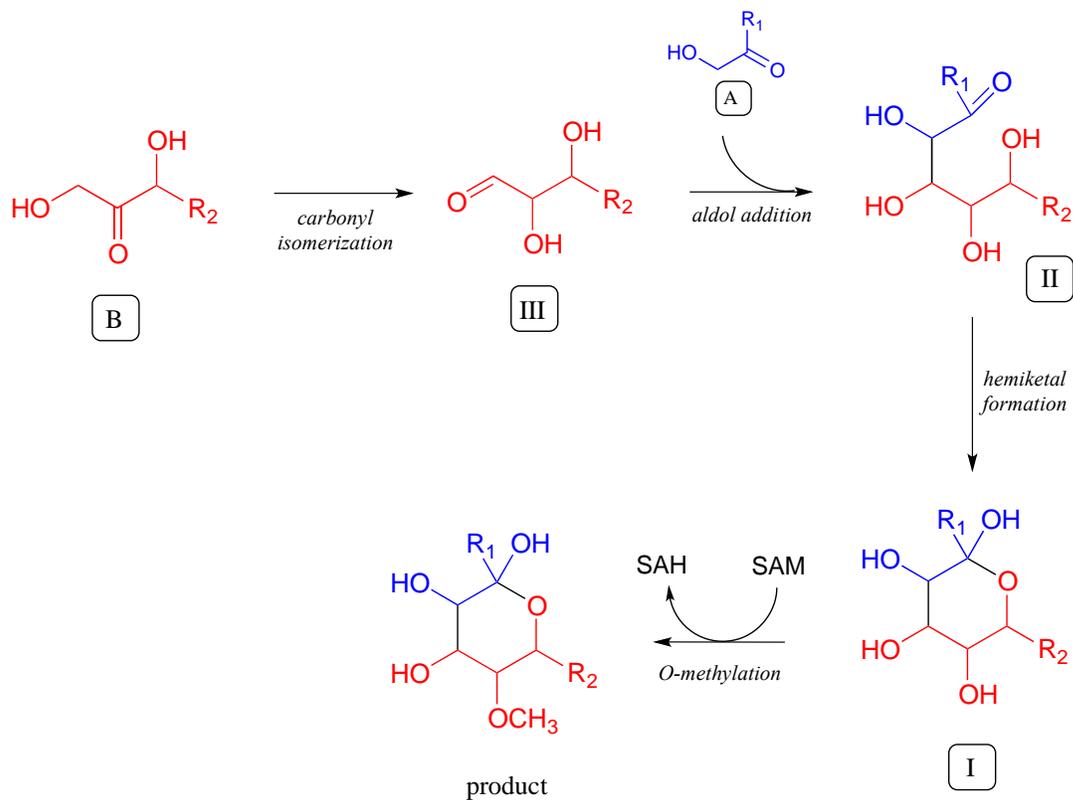
Thus, *the next disconnection is between carbons b and c*. Here's where our mastery of biological organic reactivity really comes into play: the OH at carbon c of intermediate II is in the β position relative to carbonyl carbon a. Aldol addition reactions (section 12.3) result in β -hydroxy ketones or aldehydes. Therefore, we can work backward one more step and predict that our intermediate II was formed from an aldol addition reaction between intermediate III (as the nucleophile) and precursor molecule A (as the electrophile).



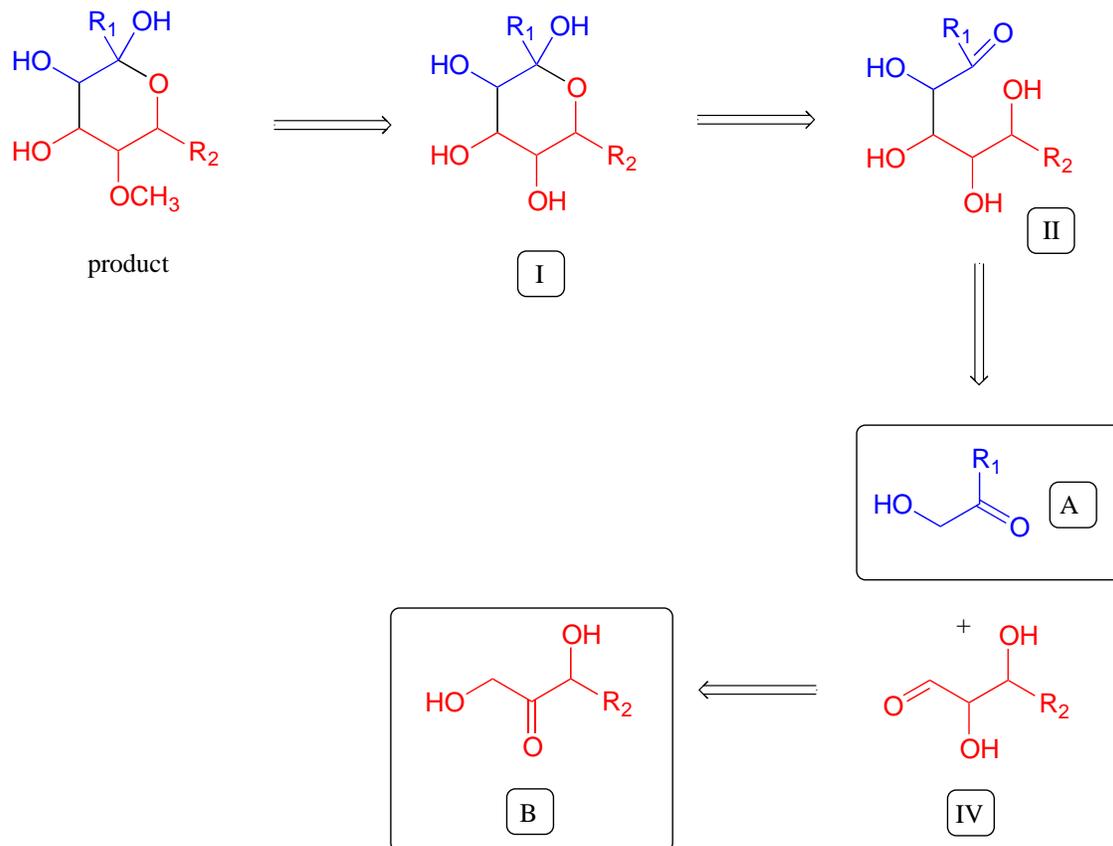
We are most of the way home - we have successfully accounted for given precursor A. Intermediate III, however, is *not* precursor B. What is different? Both III and B have a carbonyl and two alcohol groups, but the positioning is different: III is an aldehyde, while B is a ketone. Think back to earlier in this chapter: intermediate III could form from isomerization of the carbonyl group in compound B (section 12.2A). We have now accounted for our second precursor - we are done!



In the forward direction, a complete pathway diagram can be written as follows:



A full 'retrosynthesis' diagram for this problem looks like this:



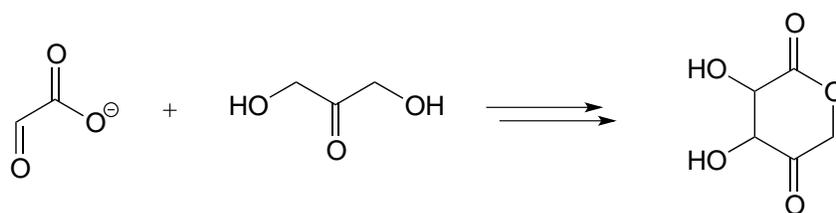
In the multi-step pathway prediction problems that you will be asked to solve below and in the remainder of this book, you will be instructed to present your solution in the form of a proposed 'forward' pathway diagram, showing the participation of all coenzymes and other species such as water. At first, we'll start with relatively simple, hypothetical biochemical transformations. As you learn more reaction types in chapter 13-17, the range and complexity of problems that you will be able to solve will expand correspondingly, and you will eventually be able to tackle real-life pathways.

Problems

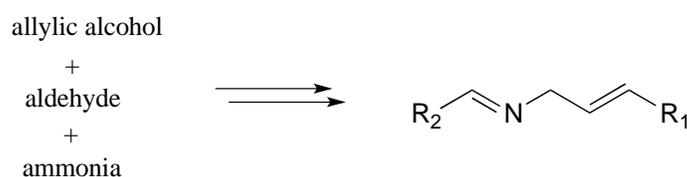
For each transformation below, draw a pathway diagram illustrating a potential biosynthetic pathway. Indicate other molecules participating in the reaction but not shown below (eg. coenzymes, water, etc.). Each step should be recognizable as a reaction type that we have covered through the end of chapter 12. (Note - you are being asked to draw your pathways in the 'forward' direction, but you should attack each problem using a retrosynthetic analysis strategy).

Solutions to these problems are available in 'Solutions to In-Chapter Exercises'.

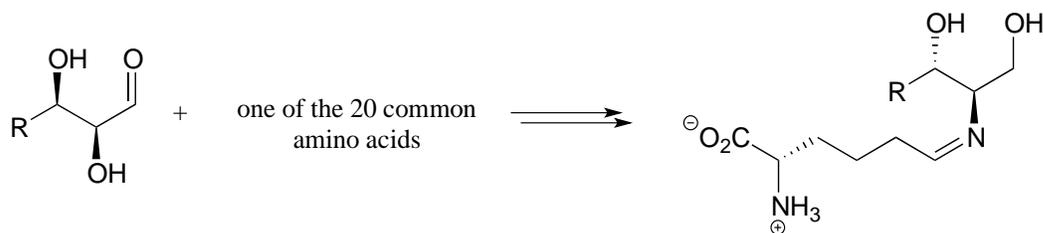
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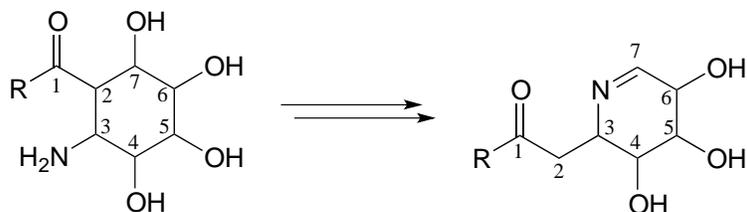
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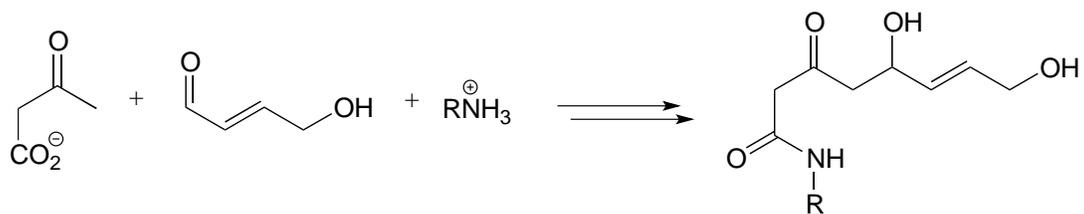
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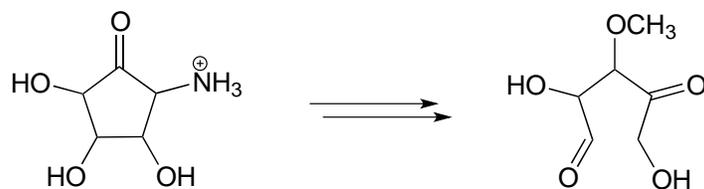
4:



5:



6:



Chapter 13

Reactions at the α -carbon, part II



Photo credit: <http://www.mixedfitness.com/>

Introduction

We begin this chapter with the story of two men, and two chemical reactions.

The two men couldn't be more different. One was an acclaimed scientist who lived and continued to work productively into his eighties. The other was struck down as a young boy by what was assumed at the time to be a fatal disease. With the heroic support of his parents and caregivers, though, he lived to his thirtieth birthday and provided the inspiration for development of a medical treatment that could potentially save thousands of lives.

The two chemical reactions in this story are closely related, and both involve the metabolism of fats in the human body. One serves to build up fatty acid chains by repeatedly linking together two-carbon units, while the other does the reverse, progressively breaking off two-carbon pieces from a long chain fatty acid molecule. The life and work of the two men are inextricably linked to the two reactions, and while we will be learning all about the reactions in the main part of this chapter, we'll begin with the stories of the two men.

On a Saturday in January of 2007, Dr. Hugo Moser passed away in the Johns Hopkins Hospital in Baltimore, succumbing to pancreatic cancer. He was 82 years old. A neurologist who had taught and researched for much of his career at Johns Hopkins, he was well known for his workaholic nature: he had signed off on his last grant application while on the way to the hospital for major surgery just a few months previously. Two days after his death, his wife and colleague Ann Moser was back in their lab, because, she said, "He gave us all a mandate to continue with the work". Dr. Moser was a highly esteemed scientist who had devoted his life to understanding and eventually curing a class of devastating neurodegenerative diseases, most notably adrenoleukodystrophy, or ALD. In his work he was careful, rational, painstaking, and relentless – a classic scientist. But in the minds of many movie fans, he became a Hollywood villain.

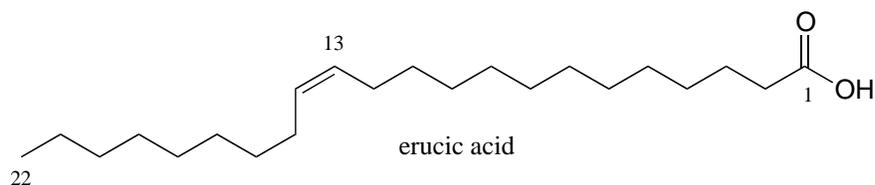
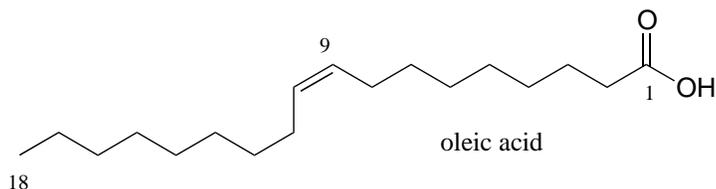
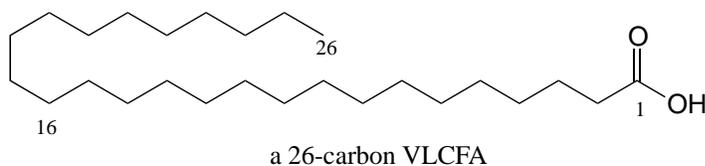
Only 17 months after the death of Dr. Moser, newspapers around the world published moving obituaries marking the passing, at age 30, of Lorenzo Odone. In one, written by his older sister and published in the British newspaper *The Guardian*, Lorenzo as a young boy is described as "lively and charming . . . he displayed a precocious gift for languages as he mastered English, Italian and French. He was funny, articulate and favored opera over nursery rhymes." But for more than 20 years leading up to his death, he had been confined to a wheelchair, blind, paralyzed, and unable to communicate except by blinking his eyes. Because he was unable to swallow, he needed an attendant to be with him around the clock to suction saliva from his mouth so he wouldn't choke.

When he was he six years old, Lorenzo started to show changes in behavior: a shortening attention span, moodiness. More disturbing to his parents, Augusto and Michaela Odone, was their suspicion that he was having trouble hearing. They took him in to be examined, and although his hearing was fine, the doctors noticed other behavioral symptoms that concerned them, and so ordered more neurological tests. The results were a kick to the stomach: Lorenzo had a fatal neurodegenerative disease called adrenoleukodystrophy. There was no cure; his nervous system would continue to degenerate, and he would probably be dead within two years.

What happened next became such a compelling story that it was eventually retold by director George Miller in the 1992 movie *Lorenzo's Oil*, starring Nick Nolte and Susan Sarandon as Augusto and Michaela Odone and Peter Ustinov as a character based on Dr. Hugo Moser. The Odones were unwilling to accept the death sentence for their son and, despite having no scientific or medical training, set about to learn everything they could about ALD.

They found out that the cause of ALD is a mutation in a gene that plays an important role in the process by which saturated fatty acids of 26 or more carbons are broken down in the body. When these 'very long chain fatty acids' (VLCFAs) accumulate to excessive levels, they begin to disrupt the structure of the myelin sheath, a protective fatty coating around nerve axons, leading eventually to degradation of the nervous system.

Researchers had found that restricting dietary intake of VLCFAs did not help – apparently much of the damage is done by the fats that are naturally synthesized by the body from shorter precursors. The Odone's realized that the key to preventing destruction of the myelin sheath might be to somehow disrupt the synthesis of VLCFAs in Lorenzo's cells. The breakthrough came when they came across studies showing that the carbon chain-elongating enzyme responsible for producing VLCFAs is inhibited by oleic and erucic acids, which are monounsaturated fatty acids of 18 and 22 carbons, respectively and are found in vegetable oils.



Administration of a mixture of these two oils, which eventually came to be known as 'Lorenzo's Oil', was shown to lead to a marked decrease in levels of VLCFAs in ALD patients.

This was, however, a therapy rather than a miracle cure – and tragically for the Odone's and the families of other children afflicted with ALD, the oil did not do anything to reverse the neurological damage that had already taken place in Lorenzo's brain. Although he was profoundly disabled, with round-the-clock care and a daily dosage of the oil Lorenzo was able to live until a day after his 30th birthday, 22 years longer than his doctors had predicted.

The story does not end there. Although the discovery of the treatment that bears his name came too late for Lorenzo Odone, might daily consumption of the oil by young children

who are at a high genetic risk for ALD possibly prevent onset of the disease in the first place, allowing them to live normal lives? This proposal was not without a lot of controversy. Many ALD experts were very skeptical of the Lorenzo's oil treatment as there was no rigorous scientific evidence for its therapeutic effectiveness, and indeed erucic oil was thought to be potentially toxic in the quantities ingested by Lorenzo. Most doctors declined to prescribe the oil for their ALD patients until more studies could be carried out. The Hollywood version of Lorenzo's story cast the medical and scientific establishment, and Dr. Hugo Moser in particular, in a strikingly negative light – they were portrayed as rigid, callous technocrats who cared more about money and academic prestige than the lives of real people. Dr. Moser was not mentioned by name in the movie, but the character played by Peter Ustinov was based closely on him: as his obituary in the Washington Post recounts, Dr. Moser once told an interviewer "The good guys were given real names. The bad guys were given pseudonyms."

What Hugo Moser in fact did was what a good scientist should always do: he kept an open mind, set up and performed careful, rigorous experiments, and looked at what the evidence told him. In a 2005 paper, Moser was finally able to confidently report his results: when young children at risk of developing ALD were given a daily dose of Lorenzo's oil, they had significantly better chance of avoiding the disease later on.

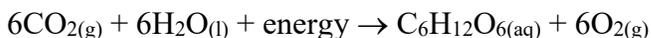
When he died, Dr. Moser was tantalizingly close to demonstrating conclusively that a simple and rapid blood test that he and his team had developed could reliably identify newborns at high risk of developing ALD – but it was not until after his death that his colleagues, including his wife, Ann Moser, were able to publish results showing that the test worked. The hope is that many lives might be saved by routinely screening newborns for ALD and responding with appropriate preventive treatments - possibly including Lorenzo's oil.

The biochemical reactions at the heart of the Lorenzo's oil story – the carbon-carbon bond forming and bond breaking steps in the synthesis and degradation of fatty acids - both involve chemistry at the α -carbon and proceed through enolate intermediates, much like the aldol and isomerization reactions we studied in chapter 11. They are known as 'Claisen condensation' and 'retro-Claisen cleavage' reactions, respectively, and represent another basic mechanistic pattern - in addition to the aldol reaction - that is ubiquitous in metabolism as a means of forming or breaking carbon-carbon bonds.

To begin this chapter, we will first learn about 'carboxylation' and 'decarboxylation' reactions, in which organic molecules gain or lose a bond to carbon dioxide, respectively, in a mechanism that is really just an extension of the aldol/retro-aldol reactions we learned about in the previous chapter. As part of this discussion, we will work through the mechanism of the carbon-fixing enzyme in plants commonly known as 'Rubisco', which is thought to be the most abundant enzyme on the planet. Then, we will move to the Claisen reactions that are so central to lipid metabolism and the story of Lorenzo Odone. Finally, we will study 'conjugate additions' and ' β -eliminations', common reaction patterns that involve double bonds in the α - β position relative to a carbonyl group, and which, again, proceed via enolate intermediates.

Section 13.1: Decarboxylation

Many carbon-carbon bond-forming and bond-breaking processes in biological chemistry involve the gain or loss, by an organic molecule, of a single carbon atom in the form of CO_2 . You undoubtedly have seen this chemical equation before in an introductory biology or chemistry class:



This of course represents the photosynthetic process, by which plants (and some bacteria) harness energy from the sun to build glucose from individual carbon dioxide molecules. The key chemical step in plants in which a carbon dioxide molecule is 'fixed' (linked to a larger organic molecule) is a **carboxylation** reaction, and is catalyzed by the enzyme ribulose 1,5-bisphosphate carboxylase, commonly known as Rubisco.

The reverse chemical equation is also probably familiar to you:

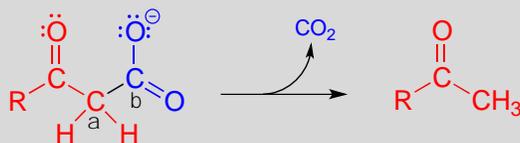


This equation expresses what happens in the process known as respiration: the oxidative breakdown of glucose to form carbon dioxide, water, and energy (in a non-biological setting, it is also the equation for the uncatalyzed *combustion* of glucose). In respiration, each of the carbon atoms of glucose is eventually converted to a CO_2 molecule. The process by which a carbon atom - in the form of carbon dioxide - breaks off from a larger organic molecule is called **decarboxylation**.

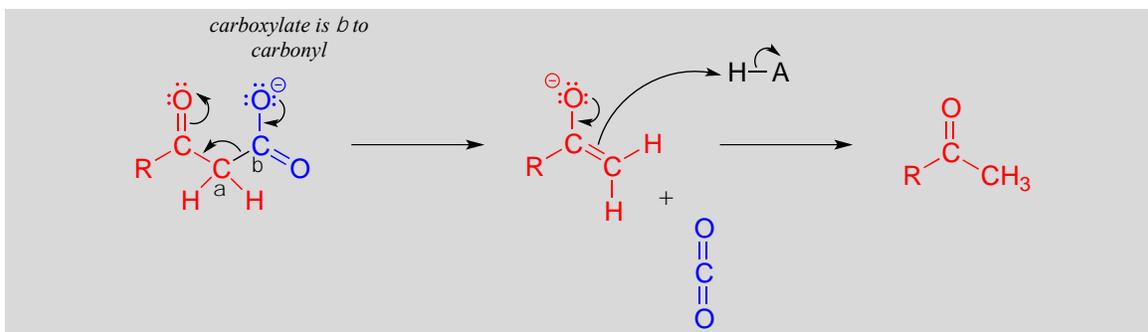
We will look now at the biochemical mechanism of decarboxylation reactions. Later in the chapter, we will look at the carboxylation reaction catalyzed by the Rubisco enzyme.

Decarboxylation steps occur at many points throughout central metabolism. Most often, the substrate for a decarboxylation step is a β -carboxy ketone or aldehyde.

Decarboxylation of a β -carboxy ketone or aldehyde:

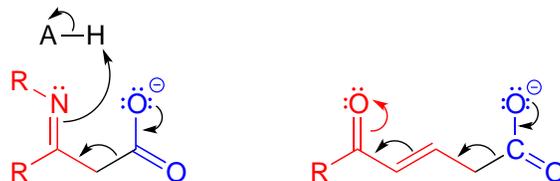


Mechanism:



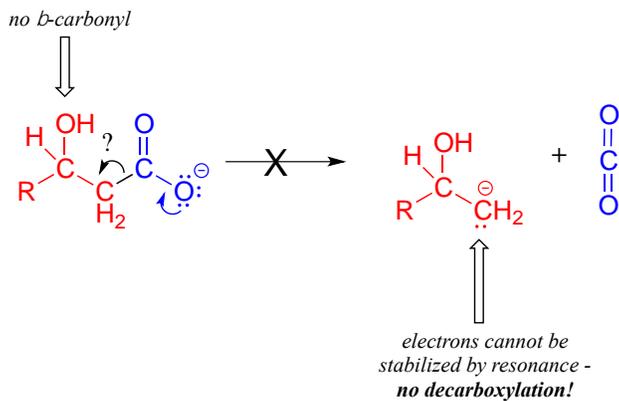
Just as in a retro-aldol reaction, a carbon-carbon bond is broken, and the electrons from the broken bond must be stabilized for the step to take place. Quite often, the electrons are stabilized by the formation of an enolate, as is the case in the general mechanism pictured above.

The electrons from the breaking carbon-carbon bond can also be stabilized by a conjugated imine group and/or by a more extensively conjugated carbonyl.



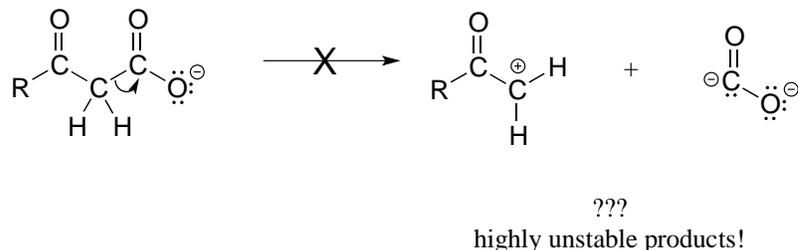
The key in understanding decarboxylation reactions is to first mentally 'push' the electrons away from the carboxylate group, then ask yourself: where do these electrons go? If the electrons cannot 'land' in a position where they are stabilized, usually by resonance with an oxygen or nitrogen, then a decarboxylation is very unlikely.

The compound below is *not* likely to undergo decarboxylation:



Be especially careful, when drawing decarboxylation mechanisms, to resist the temptation to treat the CO_2 molecule as the leaving group in a mechanistic sense:

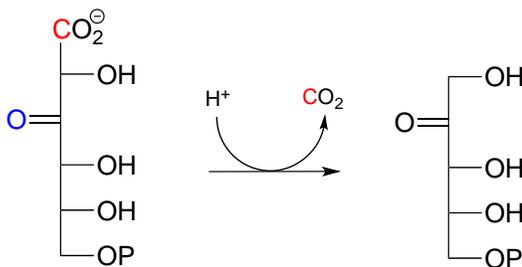
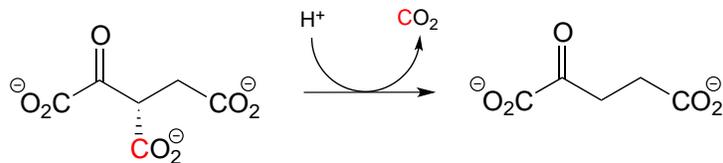
Not a decarboxylation mechanism!

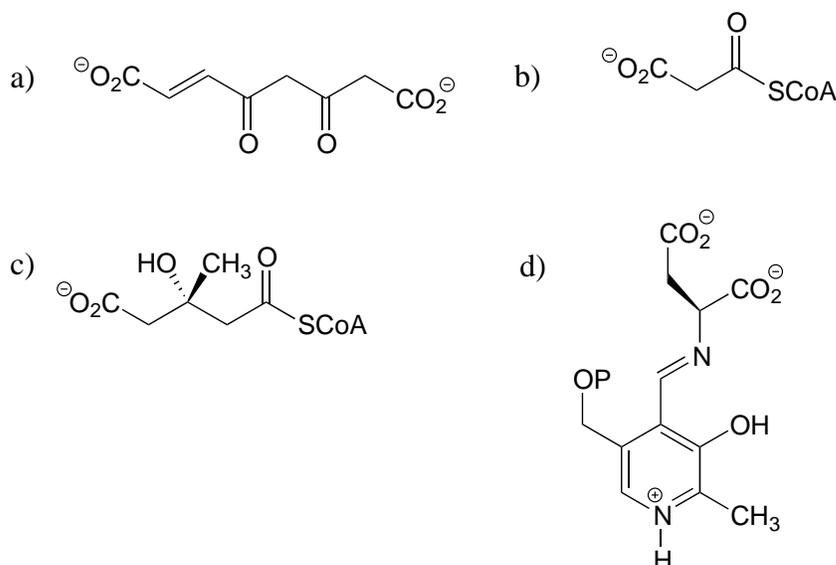


The above is *not* what a decarboxylation looks like! (Many a point has been deducted from an organic chemistry exam for precisely this mistake!) Remember that in a decarboxylation step, it is the *rest of the molecule* that is, in fact, the leaving group, 'pushed off' by the electrons on the carboxylate.

Decarboxylation reactions are generally thermodynamically favorable due to the entropic factor: one molecule is converted into two, one of which is a gas - this represents an increase in disorder (entropy). Enzymatic decarboxylation steps in metabolic pathways are also generally irreversible.

Below are two decarboxylation steps (EC 1.1.1.42; EC 1.1.1.43) in central catabolic metabolism (specifically the citric acid cycle and pentose phosphate pathway catabolism, respectively). Each step representing a point at which a carbon atom derived from the food we eat is released as carbon dioxide:





Section 13.2: An overview of fatty acid metabolism

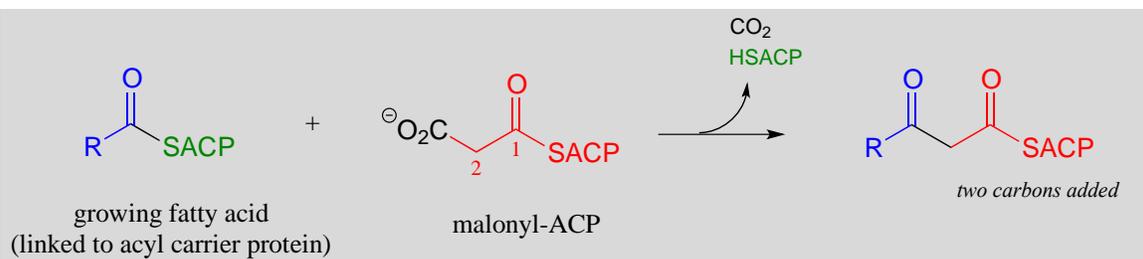
In the introduction to this chapter, we learned about a patient suffering from a rare disease affecting fatty acid metabolism. The reaction mechanisms that we are about to learn about in the next two sections are central to the process by which fatty acids are assembled (synthesis) and taken apart (degradation), so it is worth our time to go through a brief overview before diving into the chemical details.

Fatty acid metabolism is a two-carbon process: in the synthetic directions, two carbons are added at a time to a growing fatty acid chain, and in the degradative direction, two carbons are removed at a time. In each case, there is a four-step reaction cycle that gets repeated over and over. We will learn in this chapter about steps I and III in the synthesis direction, and steps II and IV in the degradative direction. The remaining reactions, and the roles played by the coenzymes involved, are the main topic of chapter 15.

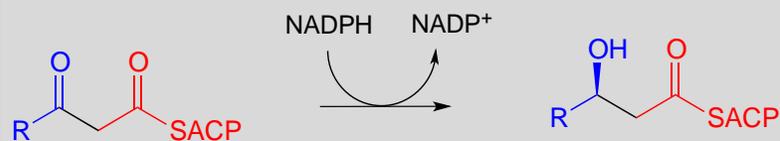
Fatty acid synthesis:

'ACP' stands for 'acyl carrier protein', which is a protein that links to growing fatty acid chains through a thioester group (see section 11.5A)

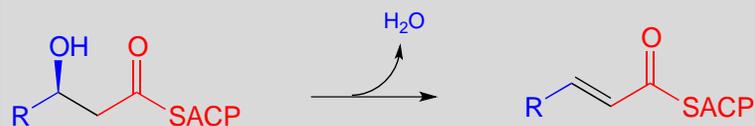
Step I: Condensation (covered in section 13.3A)



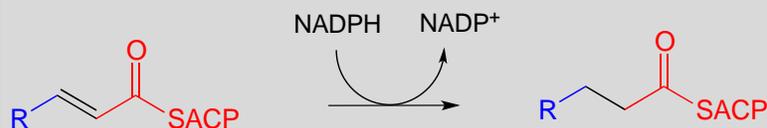
Step II: Ketone hydrogenation (covered in section 15.3)



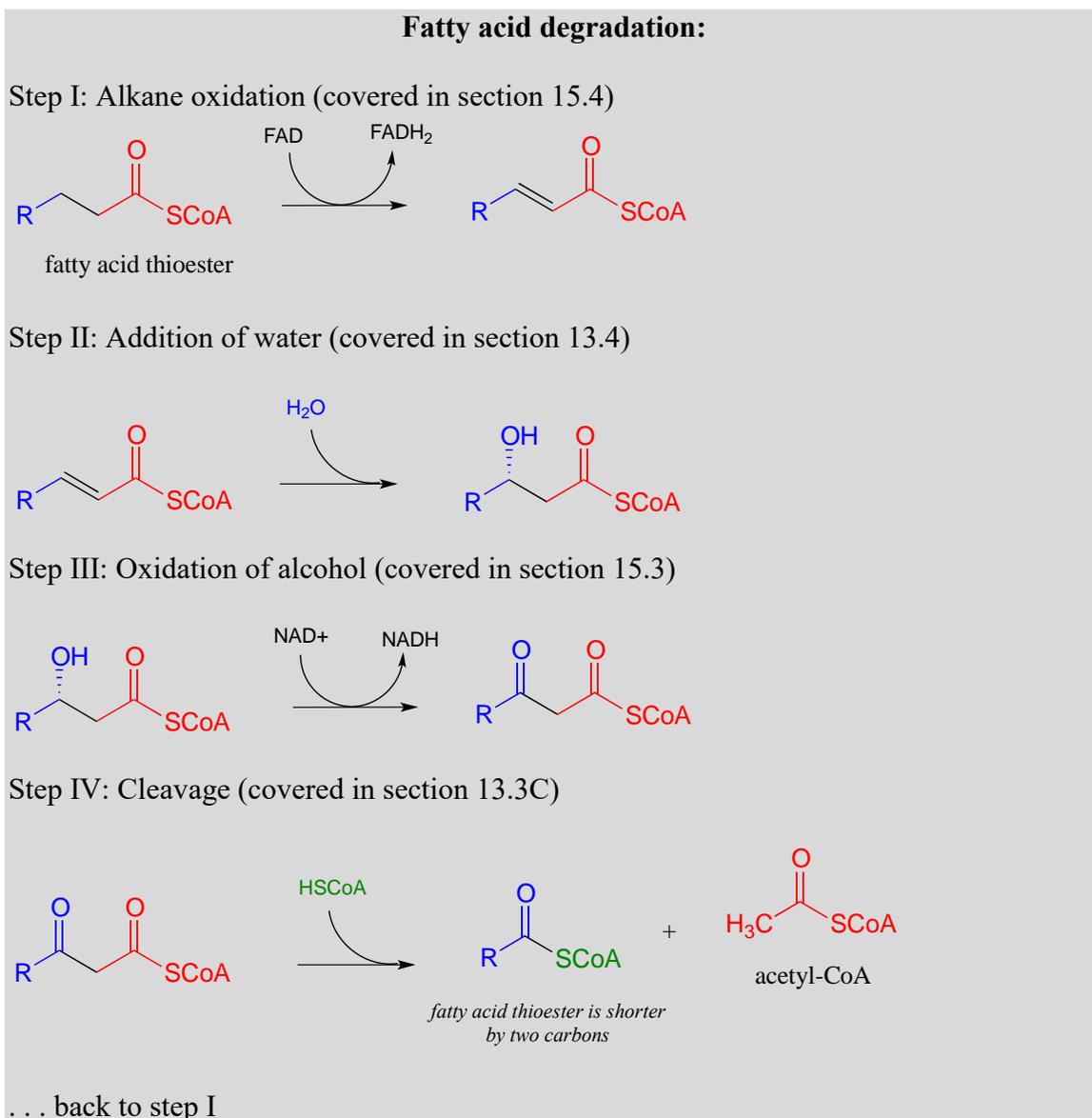
Step III: Elimination (covered in section 13.4)



Step IV: Alkene hydrogenation (covered in section 15.4)



... back to step I, add another malonyl-ACP, repeat.



When looking at these two pathways, it is important to recognize that they are *not* the reverse of each other. Different coenzymes are in play, different thioesters are involved (coenzyme A in the degradative direction, acyl carrier protein in the synthetic direction), and even the stereochemistry is different (compare the alcohols in steps II/III of both pathways). As you will learn in more detail in a biochemistry course, metabolic pathways that work in opposite directions are generally not the exact reverse of each other. In some, like fatty acid biosynthesis, all of the steps are catalyzed by different enzymes in the synthetic and degradative directions. Other 'opposite direction' pathways, such as glycolysis/gluconeogenesis, contain mostly reversible reactions (each catalyzed by one enzyme working in both directions), and a few irreversible 'check points' where the reaction steps are different in the two directions. As you will learn when you study

metabolism in biochemistry course, this has important implications in how two 'opposite direction' metabolic pathways can be regulated independently of one another.

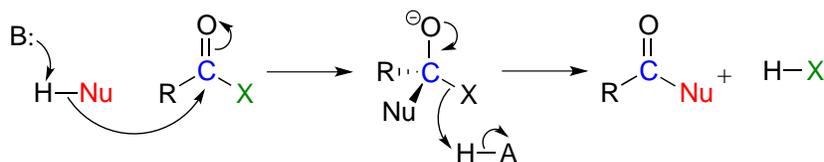
Recall that in Chapter 12 we emphasized the importance of two reaction types - the aldol addition and the Claisen condensation - in their role in forming (and breaking) most of the carbon-carbon bonds in a living cell. We have already learned about the aldol addition, and its reverse, the retro-aldol cleavage. Now, we will study the Claisen condensation reaction, and its reverse, the retro-Claisen cleavage. Step I in fatty acid synthesis is a Claisen condensation, and step IV in fatty acid degradation is a retro-Claisen cleavage.

In section 13.4, we will look more closely at the reactions taking place in step III of fatty acid synthesis (an elimination) and step II of fatty acid degradation (a conjugate addition)

Section 13.3: Claisen condensation

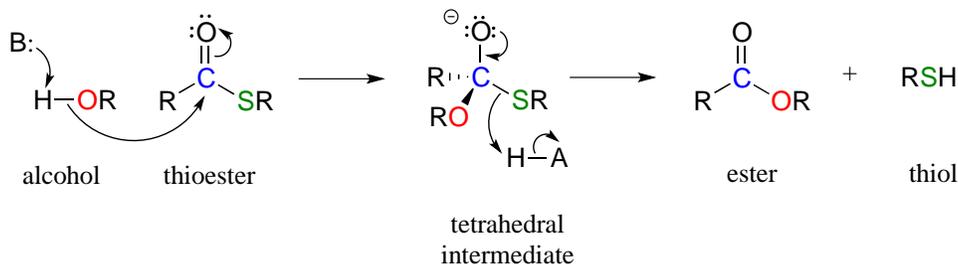
13.3A: Claisen condensation - an overview

Recall the general mechanism for a nucleophilic acyl substitution mechanism, which we studied in chapter 10:



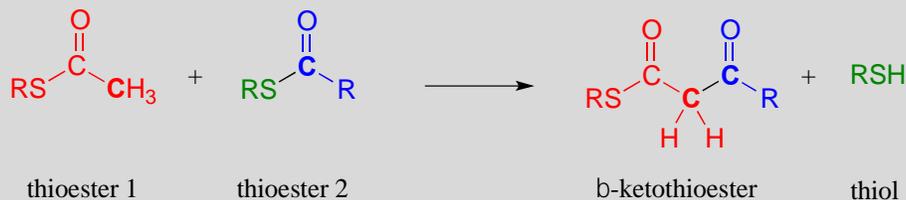
The major points to recall are that a nucleophile attacks a carboxylic acid derivative, leading to a tetrahedral intermediate, which then collapses to expel the leaving group (X). The whole process results in the formation of a different carboxylic acid derivative.

A typical nucleophilic acyl substitution reaction might have an alcohol nucleophile attacking a thioester, driving off a thiol and producing an ester.

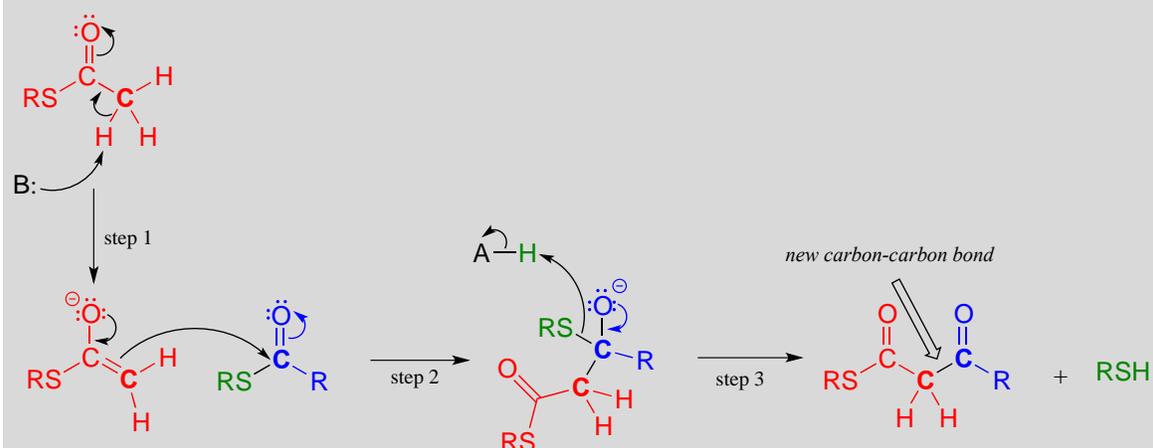


If, however, the attacking nucleophile in an acyl substitution reaction is the α -carbon of an enolate, a new carbon-carbon bond is formed. This type of reaction is called a **Claisen condensation**, after the German chemist Ludwig Claisen (1851-1930).

A Claisen condensation reaction



Mechanism:



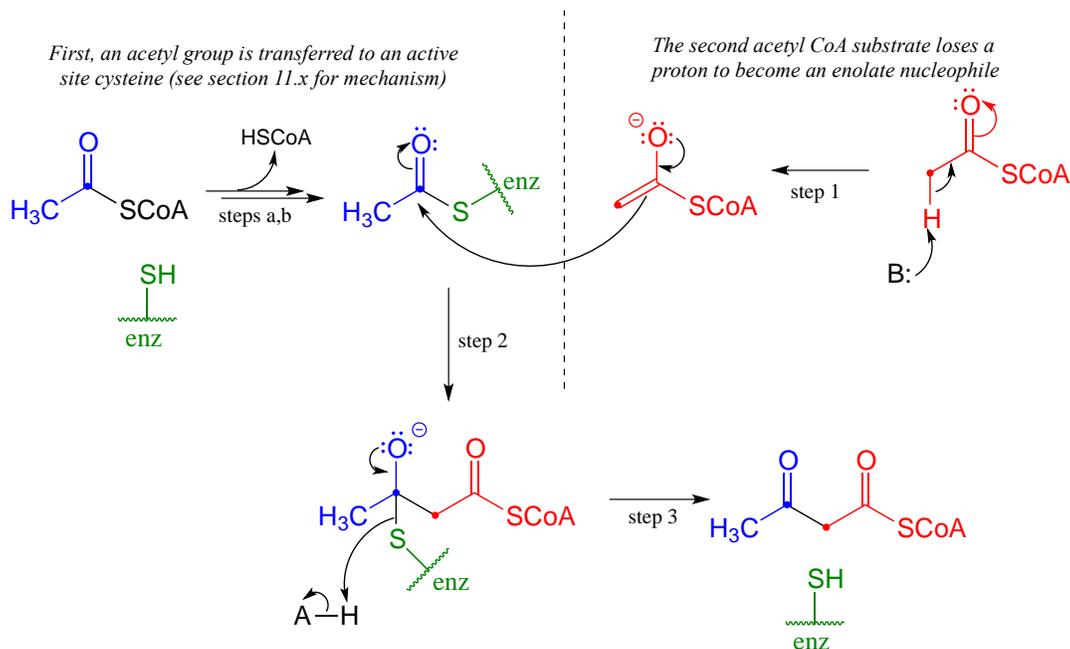
In step 1, the α -carbon of one thioester substrate is deprotonated to form an enolate, which then goes on to attack the second thioester substrate (step 2). Then the resulting tetrahedral intermediate collapses (step 3), expelling the thiol leaving group and leaving us with a β -keto thioester product (a thioester with a ketone group two carbons away).

To reiterate: *A Claisen condensation reaction is simply a nucleophilic acyl substitution (Chapter 11) reaction with an **enolate** carbon nucleophile.*

13.3B: Biochemical Claisen condensation examples

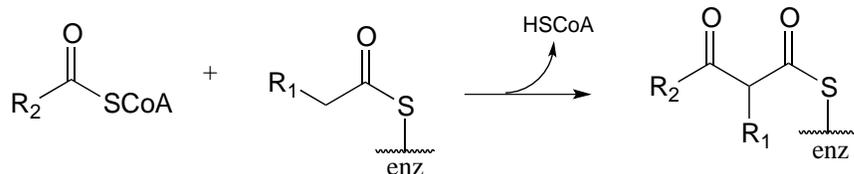
A Claisen condensation between two acetyl CoA molecules (EC 2.3.1.9) serves as the first step in the biosynthesis of cholesterol and other isoprenoid compounds in humans (see section 1.3A for a reminder of what isoprenoids are). First, a transthioesterase reaction transfers the acetyl group of the first acetyl CoA to a cysteine side chain in the enzyme's active site (steps a, b). (This preliminary event is typical of many enzyme-catalyzed Claisen condensation reactions, and serves to link the electrophilic substrate covalently to the active site of the enzyme).

In the 'main' part of the Claisen condensation mechanism, the α -carbon of a second acetyl CoA is deprotonated (step 1), forming a nucleophilic enolate.



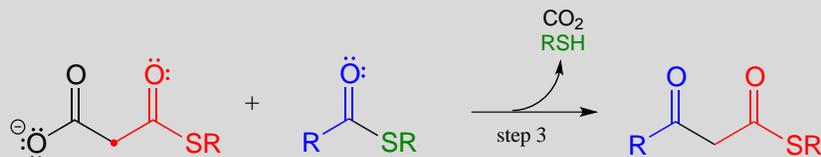
The enolate carbon attacks the electrophilic thioester carbon, forming a tetrahedral intermediate (step 2) which collapses to expel the cysteine thiol (step 3).

Exercise 13.4: Draw curved arrows for the carbon-carbon bond-forming step in mechanism for this condensation reaction between two fatty acyl-thioester substrates. R_1 and R_2 can be hydrocarbon chains of various lengths. (*J. Biol. Chem.* **2011**, 286, 10930.)



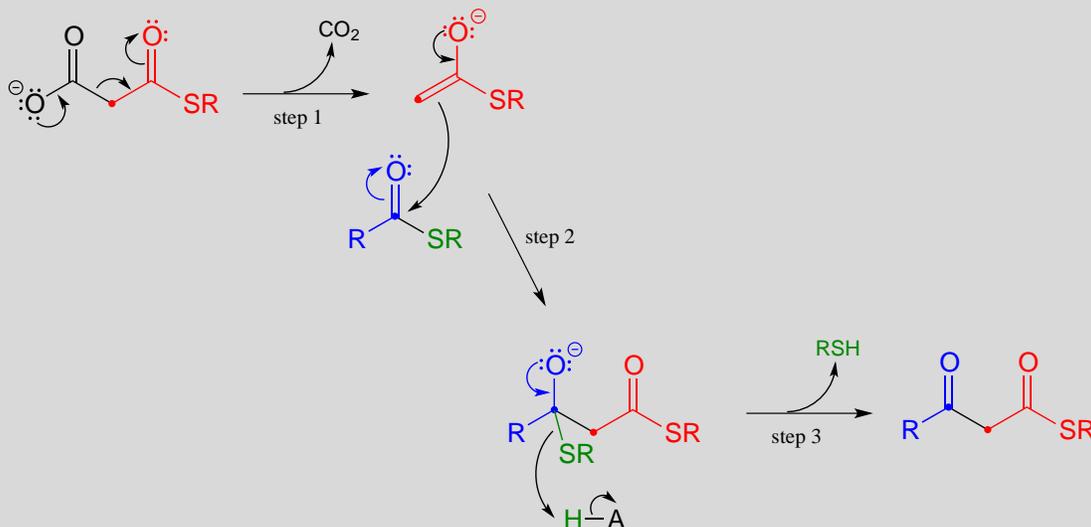
In an alternative mechanism, Claisen condensations in biology are often initiated by decarboxylation at the α -carbon of a thioester, rather than by deprotonation:

Decarboxylation/Claisen condensation:



Mechanism:

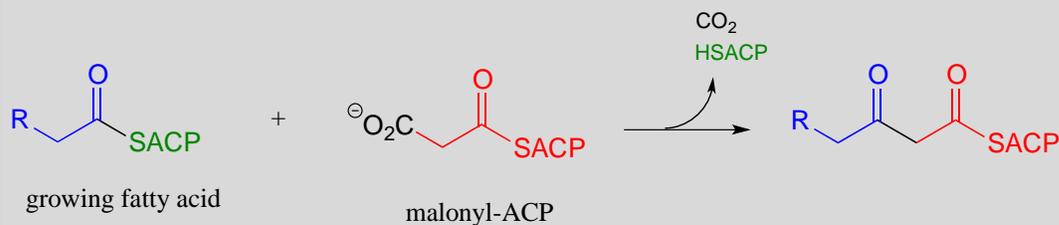
enolate nucleophile is generated by decarboxylation (instead of deprotonation)



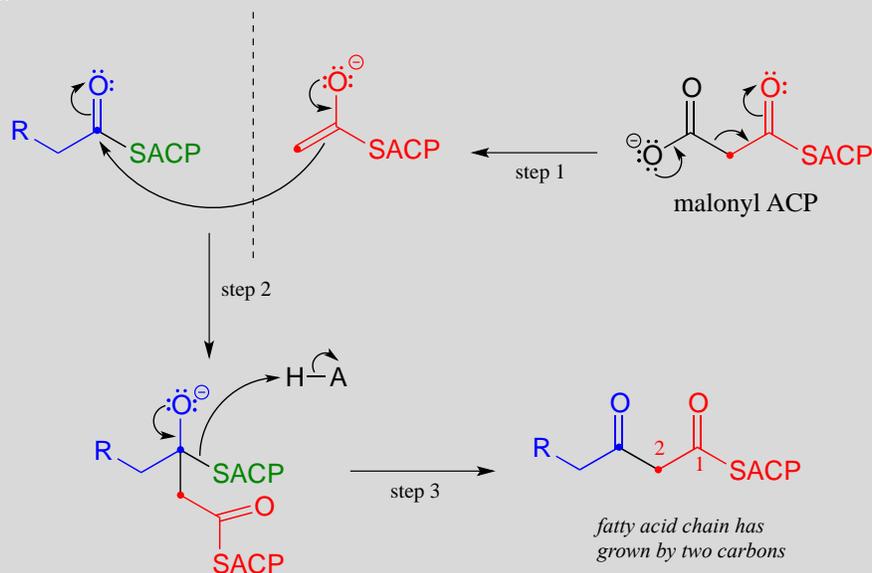
The thing to notice here is that the nucleophilic enolate (in red) is formed in the first step by decarboxylation, rather than by deprotonation of an α -carbon. Other than that, the reaction looks just like the Claisen condensation reactions we saw earlier.

Now, we can finally understand the fatty acid chain-elongation step that we heard about in the chapter introduction in the context of the Lorenzo's oil story, which is a decarboxylation/Claisen condensation between malonyl-ACP (the donor of a two-carbon unit) and a growing fatty acyl CoA molecule. Notice that, again, the electrophilic acyl group is first transferred to an active site cysteine, which then serves as the leaving group in the carbon-carbon bond forming process.

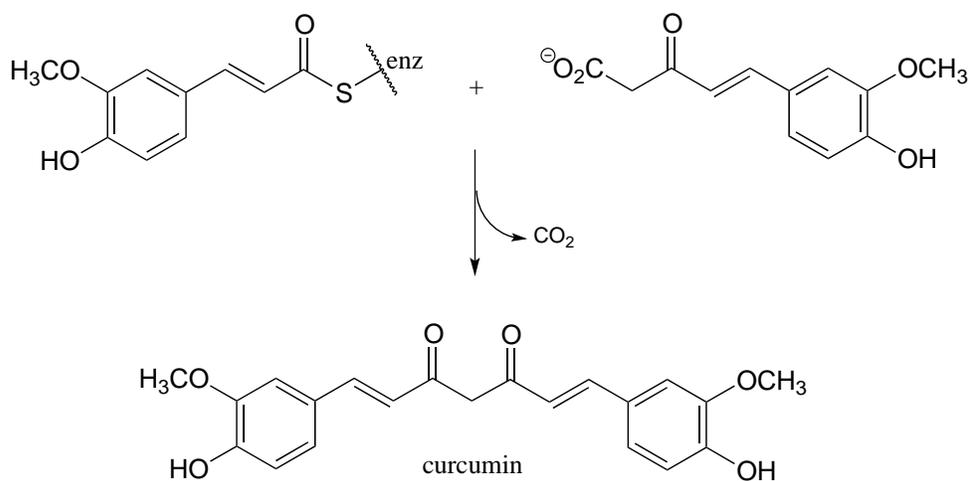
Chain elongating (Claisen condensation) reaction in fatty acid biosynthesis
(step 1 in fatty acid synthesis cycle)



Mechanism:



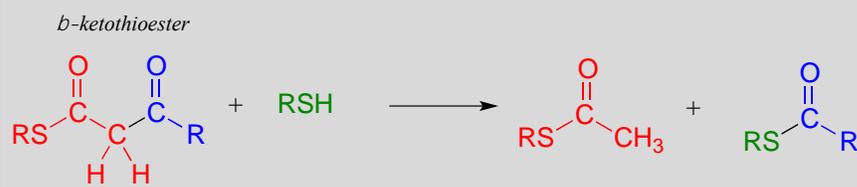
Exercise 13.5: Curcumin is the compound that is primarily responsible for the distinctive yellow color of turmeric, a spice used widely in Indian cuisine. The figure below shows the final step in the biosynthesis of curcumin. Draw a mechanism for this step.



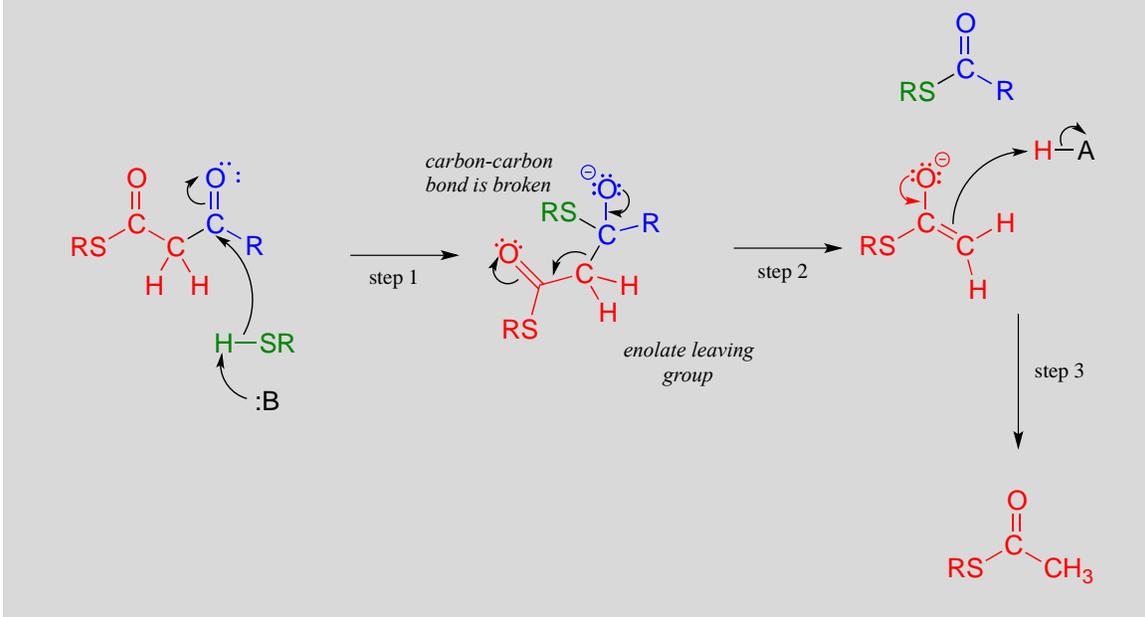
13.3C: Retro-Claisen cleavage

Just like the aldol mechanism, Claisen condensation reactions often proceed in the 'retro', bond-breaking direction in metabolic pathways.

A typical Retro-Claisen cleavage reaction
(thiol nucleophile)



Mechanism:

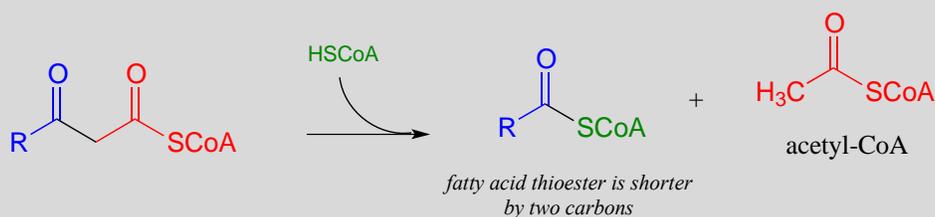


In a typical retro-Claisen reaction, a thiol (or other nucleophile such as water) attacks the carbonyl group of a β -thioester substrate (step 1), and then the resulting tetrahedral intermediate collapses to expel an enolate leaving group (step 2) - this is the key carbon-carbon bond-breaking step. The leaving enolate reprotonates (step 3) to bring us back to where we started, with two separate thioesters. You should look back at the general mechanism for a forward Claisen condensation and convince yourself that the retro-Claisen mechanism illustrated above is a step-by-step reverse process.

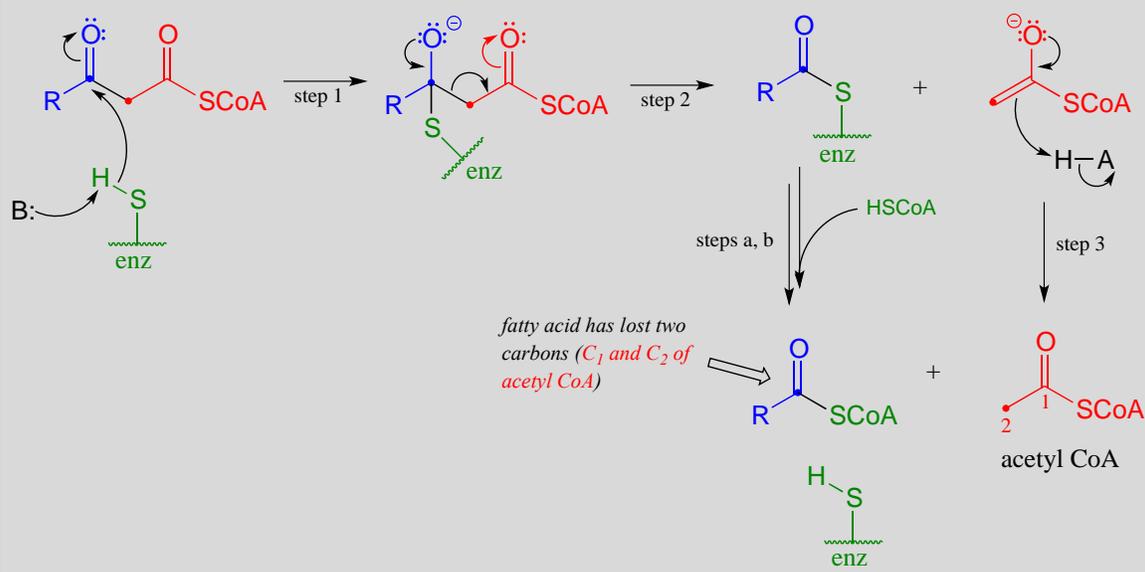
Exercise 13.6: Is a decarboxylation/Claisen condensation step also likely to be metabolically relevant in the 'retro' direction? Explain.

When your body 'burns' fat to get energy, it is a retro-Claisen cleavage reaction (EC 2.3.1.16) that is responsible for breaking the carbon-carbon bonds in step IV of the fatty acid degradative pathway. A cysteine thiol on the enzyme serves as the incoming nucleophile (step 1 in the mechanism below), driving off the enolate leaving group as the tetrahedral intermediate collapses (step 2). The enolate is then protonated to become acetyl CoA (step 3), which goes on to enter the citric acid (Krebs) cycle. Meanwhile, a transthioesterification reaction occurs (steps a and b) to free the enzyme's cysteine residue, regenerating a fatty acyl CoA molecule which is two carbons shorter than the starting substrate.

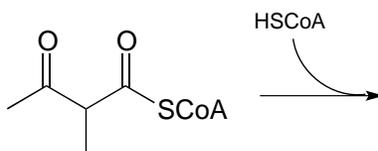
The retro-Claisen reaction (step IV) in fatty acid degradation



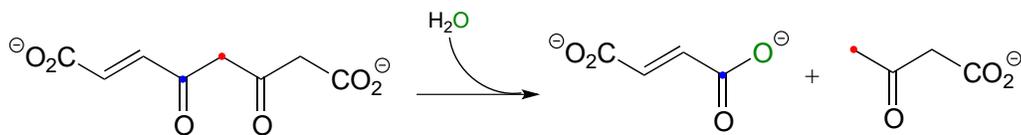
Mechanism:



Exercise 13.7: In a step in the degradation of the amino acid isoleucine, the intermediate compound 2-methyl-3-keto-butyryl CoA undergoes a retro-Claisen cleavage. Predict the products.



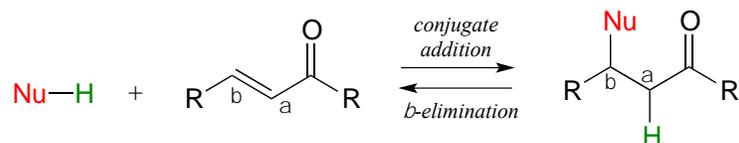
Exercise 13.8: Many biochemical retro-Claisen steps are hydrolytic, meaning that water, rather than a thiol as in the example above, is the incoming nucleophile that cleaves the carbon-carbon bond. One example (EC 3.7.1.2) occurs in the degradation pathway for tyrosine and phenylalanine:



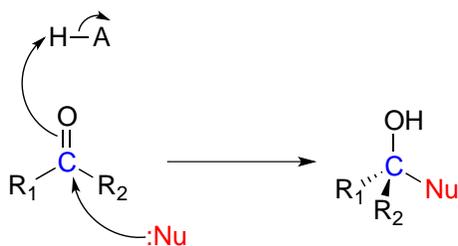
- a) Propose a likely mechanism for the reaction shown.
- b) The β -diketone substrate in the reaction above could hypothetically undergo a different retro-Claisen cleavage reaction in which the nucleophilic water attacks the other ketone group. Predict the products of this hypothetical reaction.

Section 13.4: Conjugate addition and β -elimination

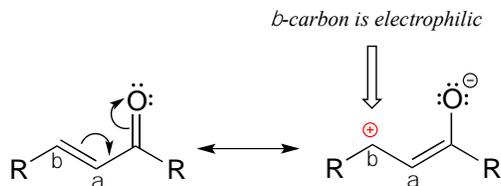
In this section, we will look at two more common biochemical reactions that proceed through enolate intermediates. In a typical **conjugate addition**, a nucleophile and a proton are 'added' to the two carbons of an alkene which is conjugated to a carbonyl (i.e. in the α - β position). In a **β -elimination** step, the reverse process occurs:



In chapter 9 we learned about nucleophilic carbonyl addition reactions, including the formation of hemiacetals, hemiketals, and imines. In all of these reactions, a nucleophile *directly* attacks a carbonyl carbon.

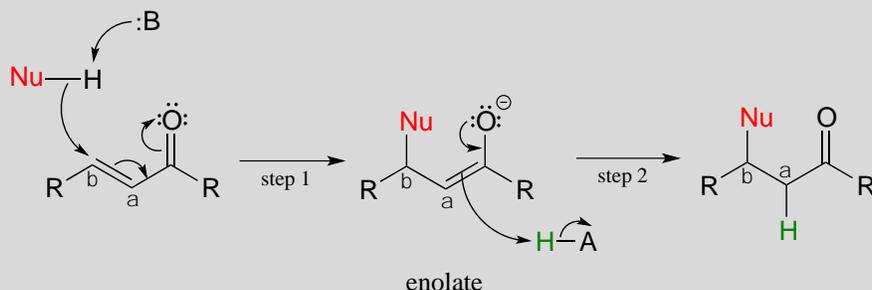


If, however, the electrophilic carbonyl is β -unsaturated - if, in other words, it contains a double bond conjugated to the carbonyl - a different reaction pathway is possible. A resonance structure can be drawn in which the β -carbon has a positive charge, meaning that the β -carbon also has the potential to be an electrophilic target.



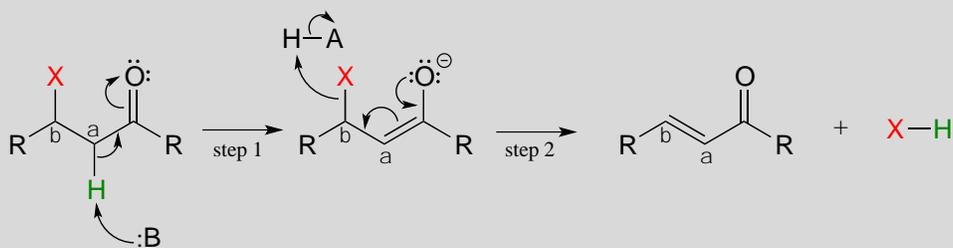
If a nucleophile attacks at the β -carbon, an enol or enolate intermediate results (step 1 below). In many cases this intermediate collapses and the α -carbon is protonated (step 2). This type of reaction is known as a **conjugate addition**.

Mechanism of a conjugate addition reaction



The reverse of a conjugate addition is a **β -elimination**, and is referred to mechanistically the abbreviation **E1cB**.

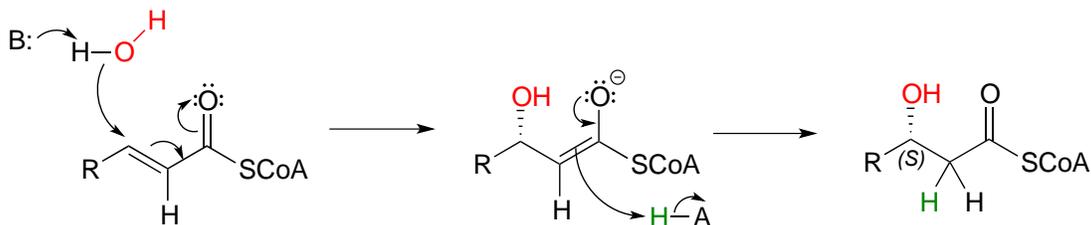
Mechanism of an E1cB elimination



The E stands for 'elimination'; the numeral 1 refers to the fact that, like the S_N1 mechanism, it is a stepwise reaction with first order kinetics. The 'cB' designation refers to the intermediate, which is the **conjugate base** of the starting compound. In step 1, an α -carbon is deprotonated to produce an enolate, just like in aldol and Claisen reactions we have already seen. In step 2, the excess electron density on the enolate expels a leaving group at the β position (designated 'X' in the figure above). Notice that the α and β carbons change from sp^3 to sp^2 hybridization with the formation of a conjugated double bond.

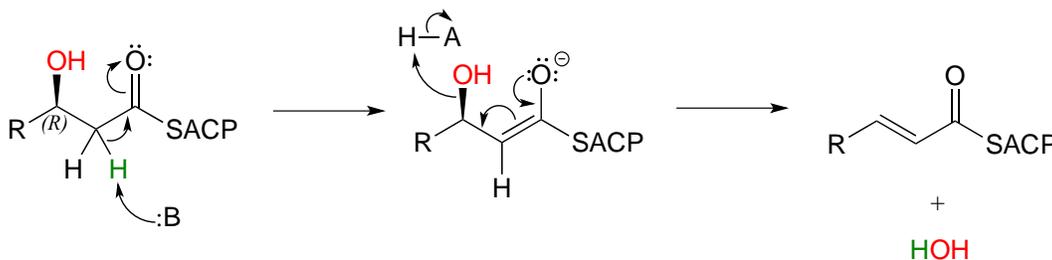
(In chapter 14 we will learn about alternate mechanisms for alkene addition and β -elimination reactions in which there is *not* an adjacent carbonyl (or imine) group, and in which the key intermediate species is a resonance-stabilized carbocation.)

Step II of fatty acid degradation is a conjugate addition of water, or hydration.



Note the specific stereochemical outcome: in the active site, the nucleophilic water is bound *behind* the plane of the conjugated system (as drawn in the figure above), and the result is *S* configuration in the β -hydroxy thioester product.

In step III of the fatty acid *synthesis* cycle we saw an E1cb β -elimination of water (**dehydration**):



Notice that the stereochemistry at the β -carbon of the starting alcohol is *R*, whereas the hydration pathway (step II) reaction in the fatty acid degradation cycle pathway results in the *S* stereoisomer. These two reactions are *not* the reverse of one another!

Here are two more examples of β -elimination reactions, with phosphate and ammonium respectively, as leaving groups. The first, 3-dehydroquinate synthase (EC 4.2.3.4) is part of the biosynthesis of aromatic amino acids, the second, aspartate ammonia lyase (EC 4.3.1.1) is part of amino acid catabolism.

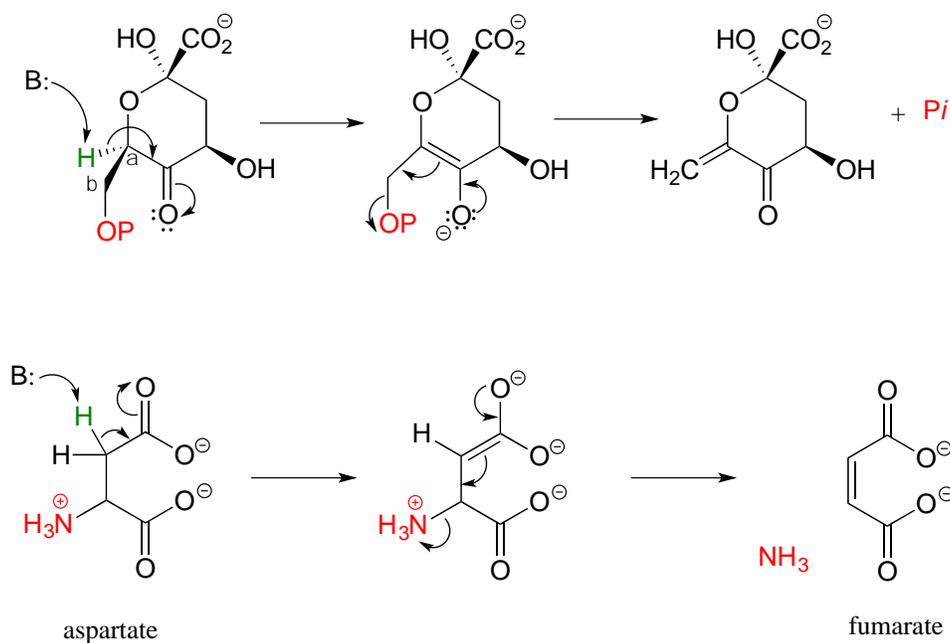
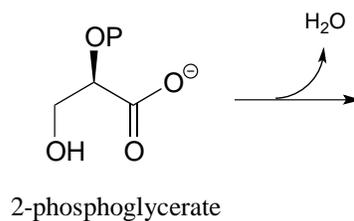
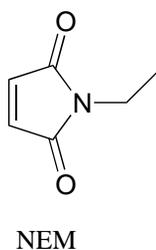


figure showing active site interactions for aspartate ammonia lyase
(*Biochemistry* 2011, 50, 6055)

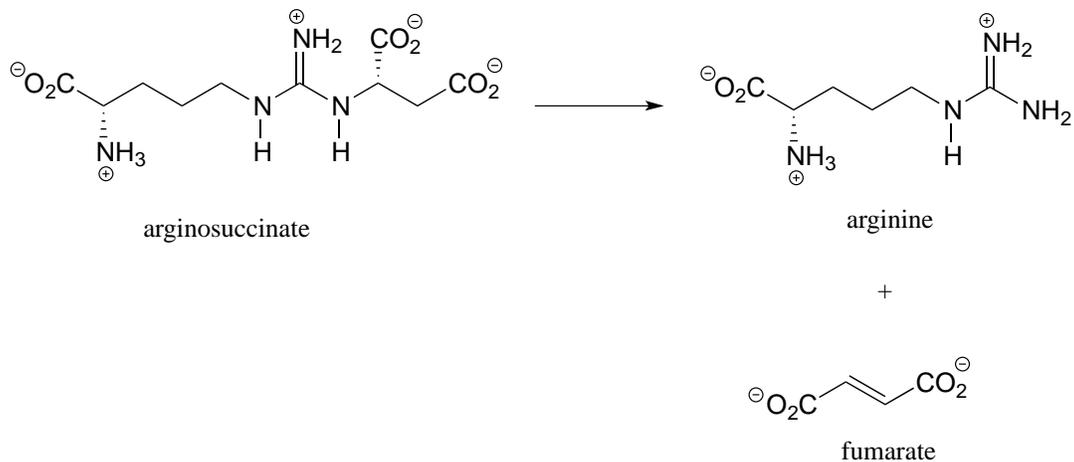
Exercise 13.9: In the glycolysis pathway, the enzyme 'enolase' (EC 4.2.1.11) catalyzes the E1cb dehydration of 2-phosphoglycerate. Predict the product of this enzymatic step.



Exercise 13.10: *N*-ethylmaleimide (NEM) is an irreversible inhibitor of many enzymes that contain active site cysteine residues. Inactivation occurs through conjugate addition of cysteine to NEM: show the structure of the labeled residue.



Exercise 13.11: Argininosuccinate lyase (4.3.2.1), an enzyme in the metabolic pathway that serves to eliminate nitrogen from your body in the form of urea in urine, catalyzes this β -elimination step:



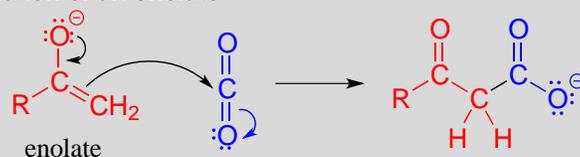
Propose a complete mechanism. *Hint:* Don't be intimidated by the size or complexity of the substrate - review the β -elimination mechanism, then identify the leaving group and breaking bond, the α -carbon which loses a proton, the carbonyl that serves to stabilize the negatively-charged (enolate) intermediate, and the double bond that forms as a result of the elimination. You may want to designate an appropriate 'R' group to reduce the amount of drawing.

Section 13.5: Carboxylation by the Rubisco enzyme

It is difficult to overstate the importance to biology and ecology of the enzymatic reaction we are going to see next: ribulose 1,5-bisphosphate carboxylase (Rubisco) plays a key role in closing the 'carbon cycle' in our biosphere, catalyzing the incorporation of a carbon atom - in the form of carbon dioxide from the atmosphere - into organic metabolites and eventually into carbohydrates, lipids, nucleic acids, and all of the other organic molecules in living things. Rubisco is probably the most abundant enzyme on the planet.

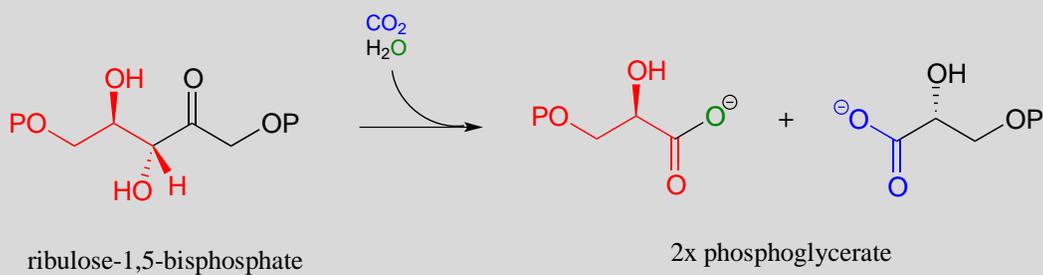
You can think of a carboxylation reaction as essentially a special kind of aldol reaction, except that the carbonyl electrophile being attacked by the enolate is CO_2 rather than a ketone or aldehyde:

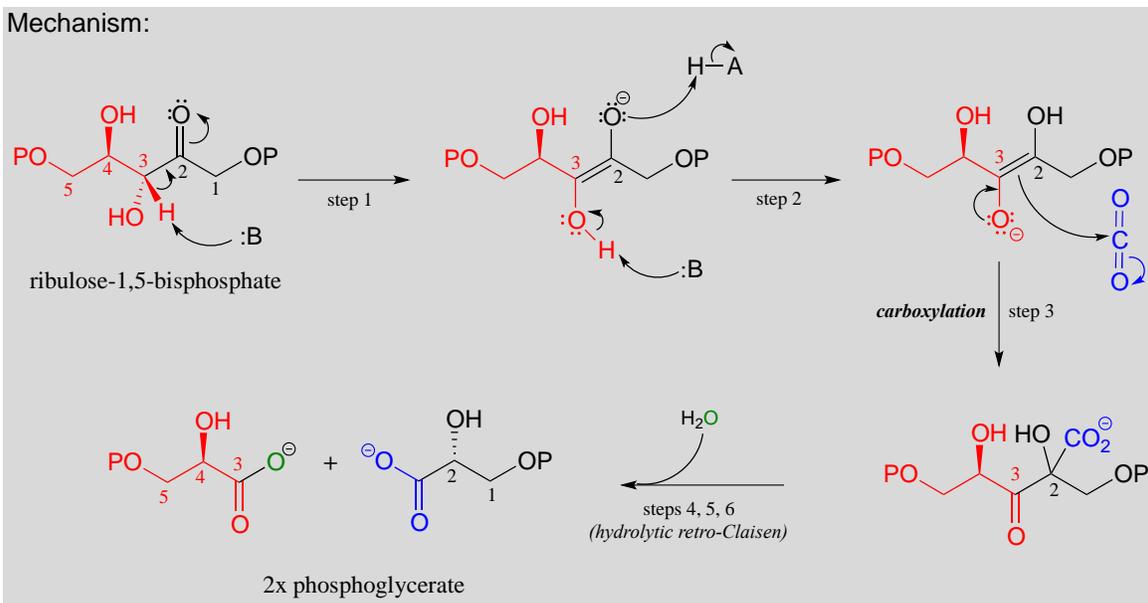
Mechanism for carboxylation of an enolate



Here is the full Rubisco reaction. Notice that the carbon dioxide (in blue) becomes incorporated into one of the two phosphoglycerate products.

The Rubisco reaction





The mechanism for the Rubisco reaction is somewhat involved, but if we break it down into its individual steps, it is not terribly difficult to follow. In step 1, an α -carbon on ribulose 1,5-bisphosphate is deprotonated to form an enolate. In step 2, the oxygen at carbon #3 is deprotonated while the oxygen at carbon #2 is protonated: combined, these two steps have the effect of creating a different enolate intermediate and making carbon #2, rather than carbon #3, into the nucleophile for an aldol-like addition to CO_2 (step 3). Carbon dioxide has now been 'fixed' into organic form - it has become a carboxylate group on a six-carbon sugar derivative. Steps 4, 5, and 6 make up a hydrolytic retro-Claisen cleavage reaction (in other words, water is the bond-breaking nucleophile) producing two molecules of 3-phosphoglycerate. Phosphoglycerate is channeled into the gluconeogenesis pathway to eventually become glucose.

Exercise 13.12: Draw out the full mechanism for steps 4-6 in the Rubisco reaction.

Key learning objectives for this chapter

Before moving on to the next chapter, you should:

Be able to draw reasonable mechanisms for reactions of the following type:

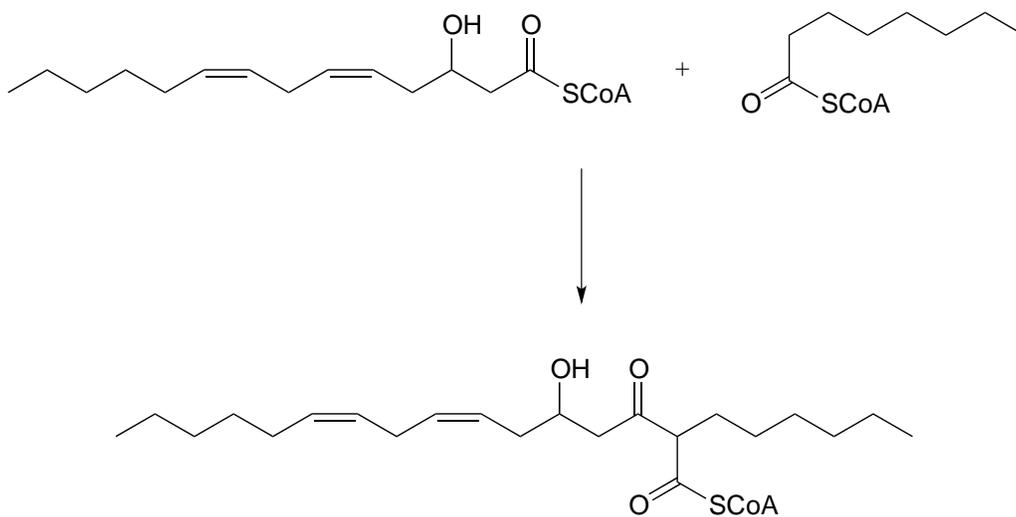
- Decarboxylation of a β -carboxy ketone or aldehyde
- Claisen condensation and retro-Claisen cleavage
- Hybrid decarboxylation-Claisen condensation
- Conjugate addition
- E1cb elimination

Understand (though not necessarily memorize) the fatty acid synthesis and degradation cycles, and how the Claisen, retro-Claisen, conjugate addition, and E1cb elimination steps fit in.

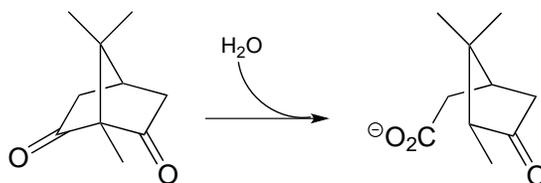
Be able to draw a complete mechanism for the Rubisco reaction.

Problems

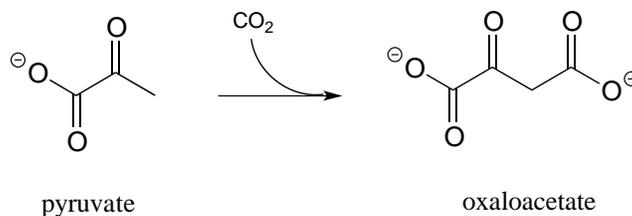
P13.1: Tetrahydrolipastatin, a potent inhibitor of lipase enzymes (see section 11.6) is being tested as a possible anti-obesity drug. Lipastatin, a close derivative, is synthesized by the bacterium *Streptomyces toxytricini*. The biosynthetic pathway involves the following step shown below - draw a likely mechanism. (*J. Biol. Chem.* **1997**, *272*, 867).



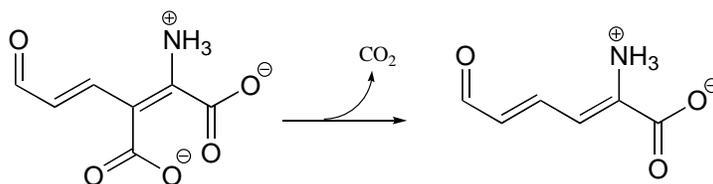
P13.2: The metabolism of camphor by some bacteria involves the step below. Draw a likely mechanism. (*J. Biol. Chem.* **2004**, *279*, 31312)



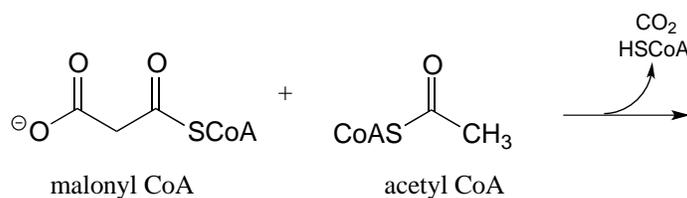
P13.3: The gluconeogenesis pathway, by which glucose is synthesized from pyruvate, begins with a reaction catalyzed by pyruvate carboxylase. The enzyme requires the CO_2 -carrying biotin to function, but the final step is thought to be the simple carboxylation of pyruvate by free carbon dioxide (*Biochem. J.* **2008**, *413*, 369). Draw a mechanism for this step.



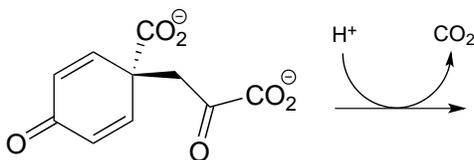
P13.4: Draw a reasonable mechanism for this decarboxylation step in tryptophan biosynthesis (EC 4.1.1.45). *Hint:* a tautomerization step precedes the decarboxylation.



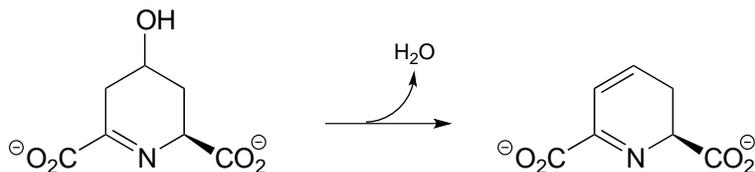
P13.5: The biosynthetic pathway for the antibiotic compound rabelomycin begins with the condensation of malonyl CoA and acetyl CoA. Predict the product of this reaction, and propose a likely mechanism. (*Org. Lett.* **2010** *12*, 2814.)



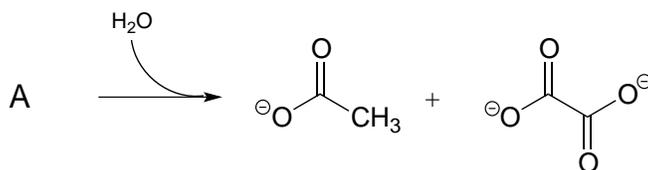
P13.6 Predict the product of this decarboxylation step in the biosynthesis of the amino acid tyrosine. *Hint:* think about comparative stability when you are considering where protonation will occur!



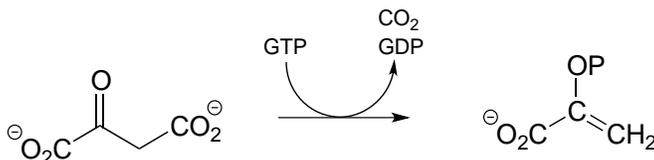
P13.7: Show a likely mechanism for this reaction from lysine biosynthesis:



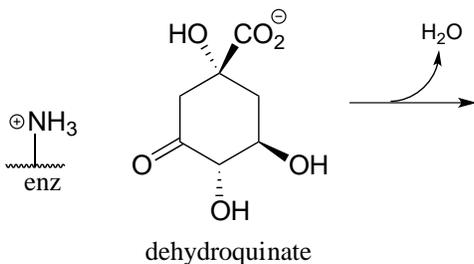
P13.8: Compound A undergoes hydrolytic cleavage in some fungi to form the products shown. Predict the structure of A. (*J. Biol. Chem.* **2007**, 282, 9581)



P13.9: Propose a mechanism for the following reaction from the gluconeogenesis pathway (EC 4.1.1.32):

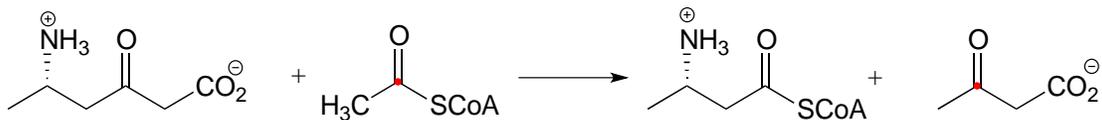


P13.10: Dehydroquinate undergoes dehydration (EC 4.2.1.10) in aromatic amino acid biosynthesis. Experimental and genomic evidence points to a lysine-linked iminium intermediate. More than one dehydration product is possible for dehydroquinate, but in this case the most stable product is the one that forms. Predict the structure of the product, explain why it is the more stable of the possible dehydration products, and draw a mechanism for its formation.

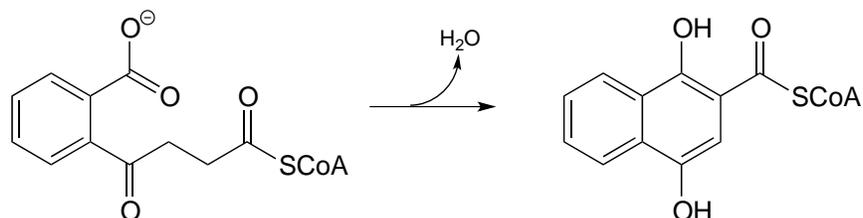


P13.11: The enzyme catalyzing the reaction below, thought to participate in the fermentation of lysine in bacteria, was recently identified and characterized (*J. Biol. Chem.* **2011**, 286, 27399). Propose a likely mechanism. Hint: the mechanism involves

two separate carbon-carbon bond forming and bond breaking steps. C₁ of acetyl CoA is identified with a red dot to help you trace it through to the product.

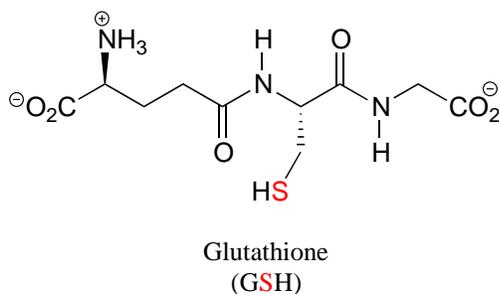
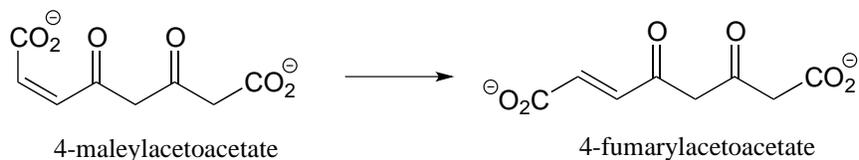


P13.12: Menaquinone (Vitamin K) biosynthesis in bacteria includes the following step:



Propose a likely mechanism. *Hint:* the mechanism involves a Claisen condensation step which is unusual in that the electrophile is a carboxylic acid group rather than a thioester. What is the driving force that allows this unusual step to occur? (*J. Biol. Chem.* **2010**, 285, 30159)

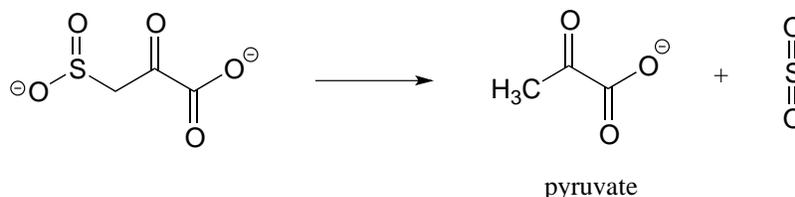
P13.13: 4-maleylacetoacetate isomerase (EC 5.2.1.2) catalyzes the following *cis* to *trans* alkene isomerization as part of the degradation of the aromatic amino acids phenylalanine and tyrosine.



The enzyme uses the thiol-containing coenzyme glutathione, which is also involved in the formation of disulfide bonds in proteins, but in this case glutathione serves as a 'thiol group for hire'. The mechanism for the reaction is essentially a reversible conjugate

addition of glutathione. Draw out the steps for this mechanism, showing how the *cis-trans* isomerization could be accomplished. Also, explain why the equilibrium for this reaction favors 4-fumarylacetoacetate. The structure of glutathione is shown, but you can use the abbreviation GSH in your mechanism.

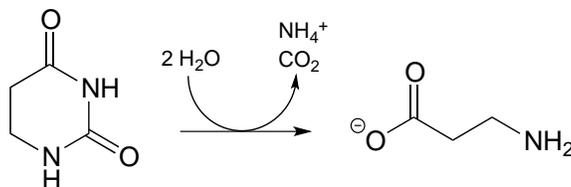
P13.14: Based on the mechanistic patterns you have studied in this chapter, propose a likely mechanism for this final reaction in the degradation of the amino acid cysteine in mammals.



P13.15: Propose a mechanism for the following carboxylation reaction (EC 6.4.1.4) in the leucine degradation pathway. The complete reaction is dependent on the CO_2 -carrying coenzyme biotin as well as ATP, but assume in your mechanism that the actual carboxylation step occurs with free CO_2 (you don't need to account for the role played by biotin or ATP).



P13.16: Propose a mechanism for the following reaction, which is part of the degradation pathway for the nucleotide uridine.

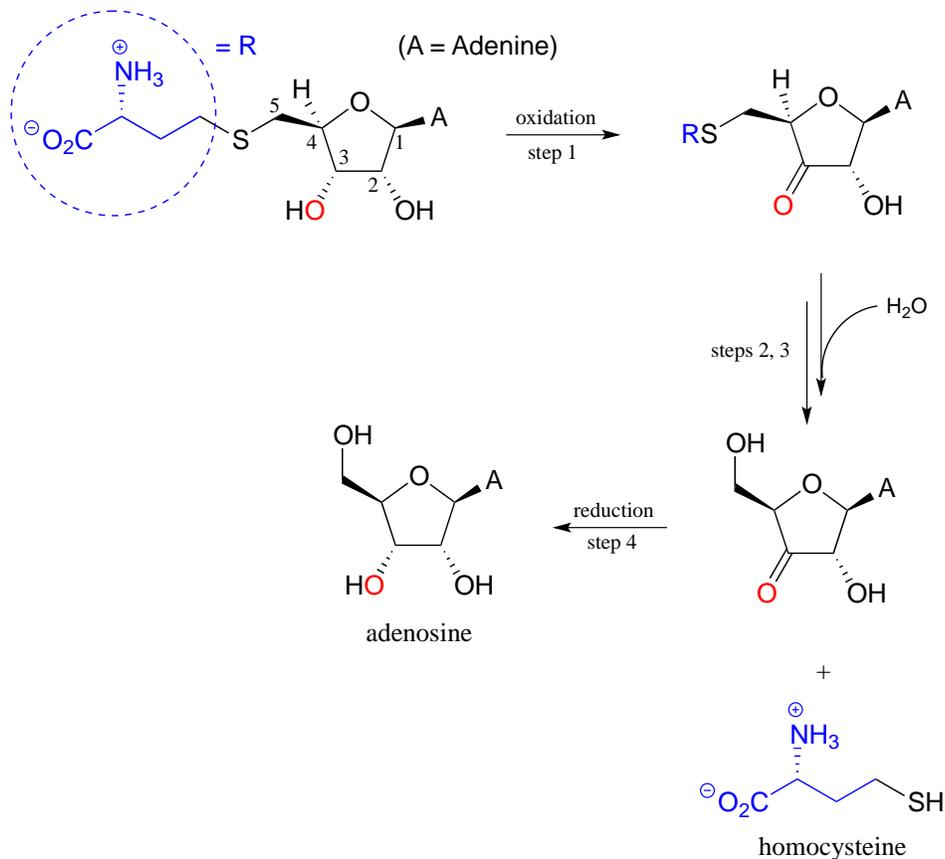


P13.17: Illustrated below is a series of reactions in the degradation pathway for the amino acid methionine. In step 1, an alcohol group on C_3 is oxidized to a ketone, and in step 4 the ketone is reduced back to an alcohol - we will study these reactions in chapter X. In steps 2 and 3, the thiol (homocysteine) is replaced by water - but this does NOT involve a nucleophilic substitution process.

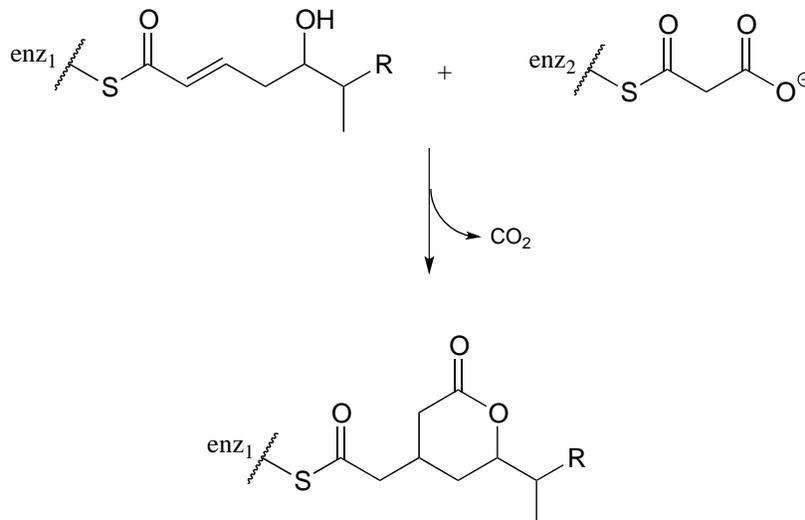
a) Draw a likely mechanism for step 2

b) Draw a likely mechanism for step 3

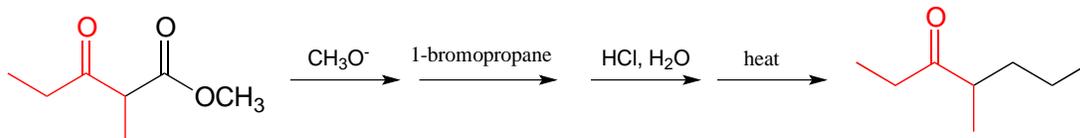
c) How does the involvement of the redox steps (steps 1 and 4) provide evidence that overall substitution of water for homocysteine is not a nucleophilic substitution?



P13.18: (Challenging!) A recently discovered reaction in the biosynthesis of rhizoxin, a potent virulence factor in the rice-seedling blight fungus *Rhizopus microsporus*, is illustrated below (*Angewandte Chemie* **2009**, 48, 5001). The reaction takes place at the intersection of two 'modules' of a multi-enzyme complex, and provides an example of a biochemical conjugate addition step that results in the formation of a new carbon-carbon bond (conjugate addition of a carbon nucleophile is referred to as a **Michael addition**). Draw a likely mechanism.

**P13.19:**

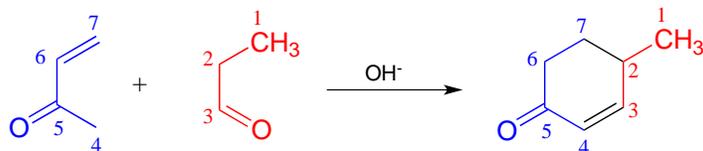
a) The 'acetoacetic ester synthesis' is a useful carbon-carbon bond-forming reaction in the laboratory. The reaction mechanism is described as α -carbon deprotonation to form an enolate, followed by $\text{S}_{\text{N}}2$ alkylation, ester hydrolysis, and decarboxylation. Below is an example:



Draw out a reasonable mechanism, taking care to propose reactive intermediates that are appropriate given the basic or acidic conditions present (note that the reaction starts under basic conditions, then is later acidified).

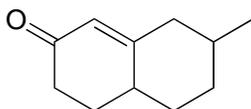
b) Suggest starting compounds for the synthesis of 4-phenyl-2-butanone by the acetoacetic ester reaction.

c) A very useful ring-forming reaction in laboratory synthesis is called 'Robinson annulation' (Sir Robert Robinson was an English chemist who won the 1947 Nobel Prize in Chemistry, and the term 'annulation' comes from the Latin *annulus*, meaning 'ring'.) The reaction, which takes place in basic conditions, consists of a conjugate (Michael) addition step, followed by aldol addition and finally dehydration (β -elimination of water). A typical example is shown below, with carbons numbered to help you to follow the course of the reaction.

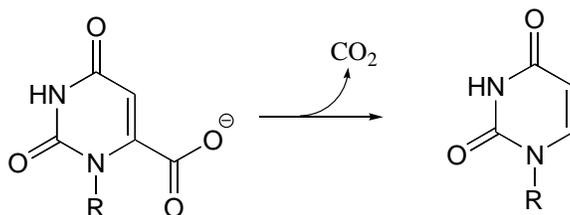


Draw a mechanism for this reaction (when proposing intermediate species, keep in mind that the reaction is occurring in a basic environment, and choose protonation states accordingly).

d) Propose starting compounds for the Robinson annulation synthesis of the following product:



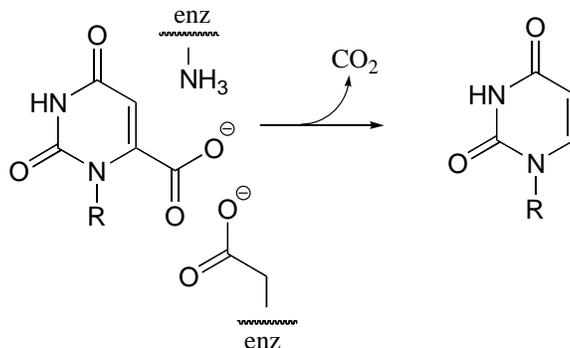
P13.20: The reaction shown below, catalyzed by orotidine monophosphate decarboxylase (EC 4.1.1.23), is one of the most extensively studied enzymatic transformations. It is known to occur without the participation of any coenzymes.



a) Look at the reaction closely: what is unique about it?

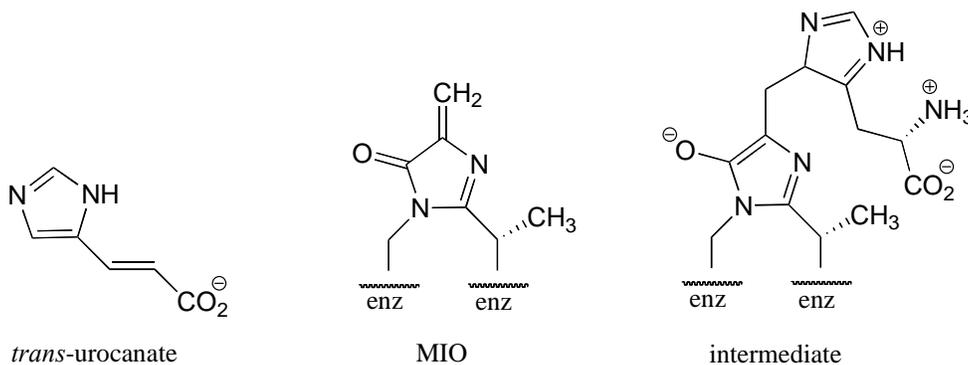
b) In 1997, a paper was published in which the authors predicted, based on theoretical calculations, that this reaction proceeded through a carbene intermediate (carbenes are not covered in this text – you may need to look them up). This was prior to the publication of an x-ray crystal structure. What kind of active site environment does this imply?

c) When the crystal structure was published a few years later, we learned that an aspartate residue (predicted to be negatively charged) is positioned very near the substrate carboxylate group, and a lysine residue (predicted to be positively charged) is positioned nearby on the opposite side (see figure below). What roles do you think were predicted for these two active site residues?



P13.21: In the histidine degradation pathway, histidine undergoes elimination of ammonia to form *trans*-urocanate. The enzyme catalyzing this reaction (E.C. 4.3.1.3) has been shown to use an unusual 'coenzyme', 4-methylideneimidazole-5-one (MIO), which is formed from the cyclization of an alanine-serine-glycine stretch of the enzyme itself.

A mechanism has been proposed in which the MIO coenzyme plays the role of electron sink, and the intermediate shown below forms.



Propose a full mechanism for this reaction according to this information.

P13.22: The product that forms in the reaction between [benzaldehyde](#) and [acetophenone](#) (along with a catalytic amount of sodium hydroxide) has a $^1\text{H-NMR}$ spectrum in which all of the signals are between 7-8 ppm. Give the structure of product.

Chapter 14

Electrophilic reactions



Memorial for Bridget Bishop, hanged as a witch in Salem, Massachusetts

photo credit: Melissa Nunez (<https://www.flickr.com/photos/143702874@N05/>)

Introduction

Linnda Caporael probably should have paid a little more attention to the graduation requirements in her college catalog. Going through the graduation checklist during her senior year, she discovered to her dismay that she still needed to fulfill a social science requirement, so she promptly enrolled in an American History course. It was a decision that would in time lead to her authoring a paper in a prestigious scientific journal, being

featured in a front page story in the New York Times, and changing our understanding of one of the most intriguing - and disturbing – episodes in American history.

Professor Caporaël (Linnda went on to become a professor of Behavioral Psychology at Rensselaer Polytechnic Institute) recounted her story in an episode of the PBS documentary series *Secrets of the Dead*. Early in the semester, she learned that as part of her history course she would be required to complete a research paper on a topic of her own choosing. She had recently seen a performance of *The Crucible*, Arthur Miller's classic play about the Salem witch trials, and decided to do her research on Anne Putnam, one of the young Salem girls who accused several village women of bewitching them. The symptoms of 'bewitchment' that afflicted the girls were truly frightening: thrashing and convulsions, visions of snakes and ferocious beasts, a sudden inability to speak, and a feeling that ants were crawling under their skin.

These children were bitten and pinched by invisible agents: their arms, necks and backs turned this way and that way, and returned back again, so as it was, impossible for them to do of themselves, and beyond the power of any epileptick fits, or natural disease to effect. Sometimes they were taken dumb, their mouth flopped, their throats choaked, their limbs wracked and tormented. . .

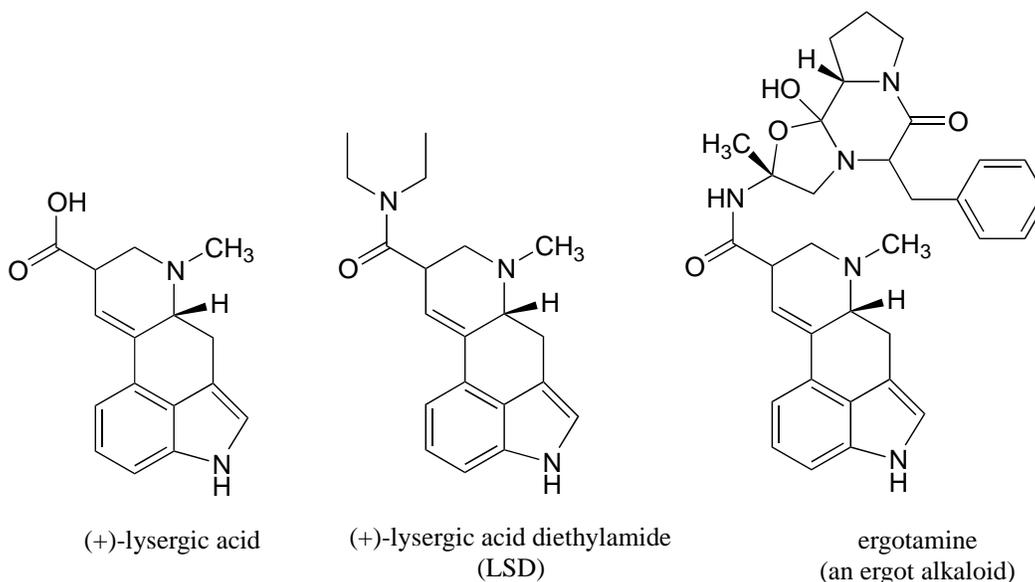
(From “A Modest Enquiry Into the Nature of Witchcraft” (1702) by Reverend John Hale. <http://historyofmassachusetts.org/betty-parris-first-afflicted-girl-of-the-salem-witch-trials/>)

As Linnda continued to read accounts of the 'fits' afflicting the Salem girls, she was suddenly struck by the similarities to another, more recent episode that she had read about. In the summer of 1951, in the French village of Pont Saint Esprit, a number of local people were simultaneously seized by hallucinations, convulsions, and other symptoms very much like those described during the Salem witch trials hundreds of years earlier. Leon Armunier, a former postman in Pont Saint Esprit, described his experience to the BBC:

It was terrible. I had the sensation of shrinking and shrinking, and the fire and the serpents coiling around my arms . . . Some of my friends tried to get out of the window. They were thrashing wildly. . . screaming, and the sound of the metal beds and the jumping up and down... the noise was terrible. I'd prefer to die rather than go through that again.

There have been several explanations offered for what caused the Pont Saint Esprit outbreak (including that the CIA was experimenting with mass LSD poisoning as a form of chemical warfare), but the most widely accepted theory is that the hallucinations were caused by eating bread made from contaminated grain.

Claviceps purpurea, a fungus known to grow in rye and other grains, produces a class of hallucinogenic compounds called 'ergot alkaloids' which are derived from lysergic acid (the hallucinogenic drug LSD is a synthetic derivative of lysergic acid). *Claviceps* thrives in damp grain, and special care must be taken to avoid contamination when storing grain grown during warm, rainy summers.



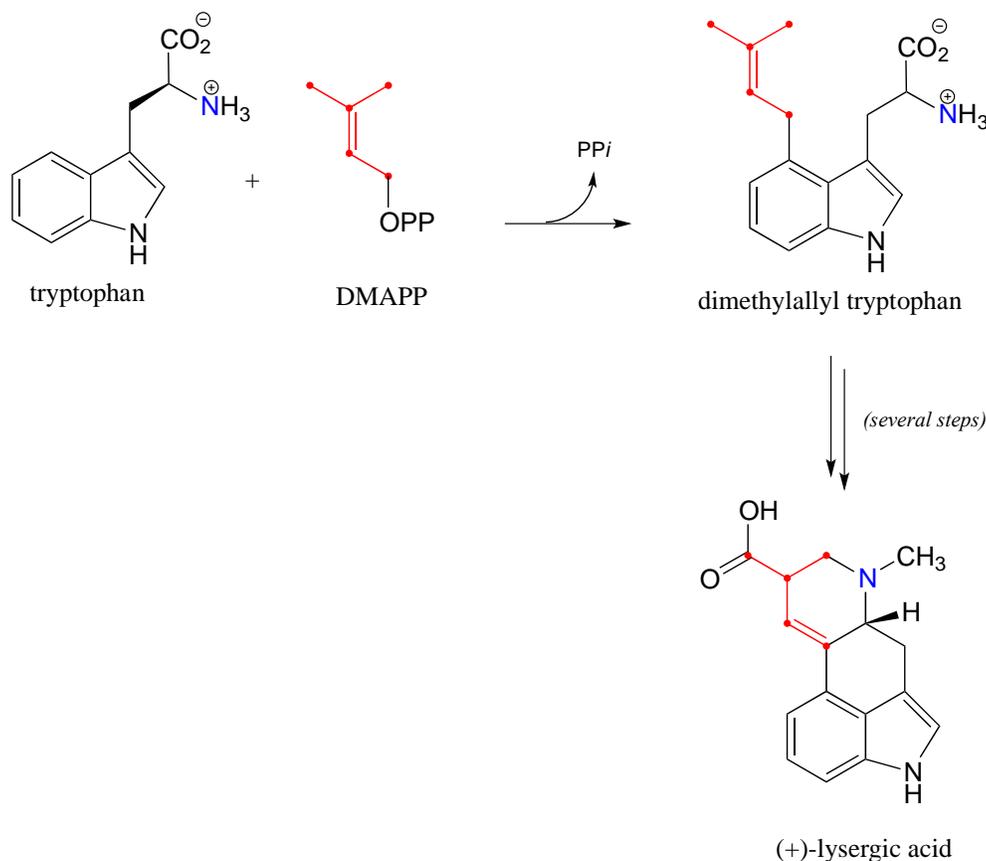
Digging deeper into the records of the Salem witchcraft episode, Linnda Caporael learned that the summer of 1691 was unusually damp. The first cases of 'bewitchment' in Salem village occurred in the winter of 1691-1692, when the villagers would have been consuming grain stored from the previous summer. Rye, the kind of grain most vulnerable to *Claviceps* contamination, was the staple crop in Salem at the time. Furthermore, nearly all of the affected girls lived on farms on the swampy western edge of the town, where *Claviceps* contamination would have been most likely to occur.

This was all circumstantial evidence, to be sure, but it was enough to convince Linnda that ergot poisoning was much more plausible as a root cause of the behavior of the afflicted girls than simply mass hysteria, which had long been the accepted theory. She summarized her findings in a paper that was later published in the journal *Science*, with the colorful title "Ergotism: The Satan Loosed in Salem?" (*Science* **1976**, 192, 21). Her theory is still not universally accepted, but scientists and historians are for the most part in agreement that ergot poisoning was the cause of other outbreaks of convulsions and hallucinations, often called 'Saint Anthony's Fire', that have occurred throughout European history. It is possible that ergot poisoning may have played a role in literature as well: professor Caporael, in an interview with PBS, recounts how she was recently contacted by an historian with an intriguing idea. Is it possible that Caliban, the wild, half-human character in Shakespeare's *The Tempest* who is tormented by hallucinations inflicted upon him by the wizard Prospero, could be a literary manifestation of ergot poisoning episodes that occurred in England during Shakespeare's time?

For every trifle are they set upon me;
 Sometime like apes that mow and chatter at me
 And after bite me, then like hedgehogs which
 Lie tumbling in my barefoot way and mount
 Their pricks at my footfall; sometime am I
 All wound with adders who with cloven tongues
 Do hiss me into madness.

(William Shakespeare's *The Tempest*, Act II scene ii)

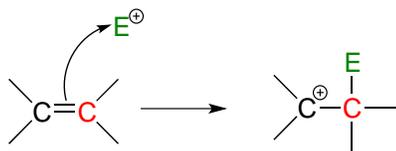
In this chapter, we will learn about a class of organic reaction that is central to the biosynthesis of ergot alkaloids in *Claviceps*. The key first step in the biosynthetic pathway is a reaction unlike any we have yet seen:



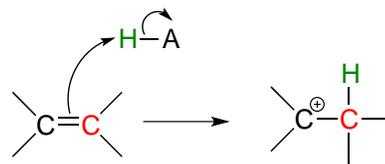
As you can see, the first step is condensation between the amino acid tryptophan and dimethylallyl diphosphate (DMAPP), the building block molecule for isoprenoids (section 1.3A). What you should also notice in the reaction figure above is that a new carbon-carbon bond is formed, and yet the chemistry involved is clearly very different from the carbon-carbon bonding forming aldol additions and Claisen condensations we learned about in the previous two chapters: there is no carbonyl to be found anywhere near the site of reaction, and one of the bond-forming carbons is part of an aromatic ring.

We will see later in this chapter that this reaction mechanism is classified as an 'electrophilic aromatic substitution', and is one of a broader family of organic reaction mechanisms that includes electrophilic additions, substitutions, and isomerizations. 'Electrophilic' is the key term here: in organic chemistry, an 'electrophilic' reaction mechanism is one in which the π -bonded electrons in a carbon-carbon double (or sometimes triple) bond are drawn towards an electron-poor species, often an acidic proton or carbocation. In essence, the π bond is acting as a nucleophile or base.

p electrons drawn toward electrophile, acting as **nucleophile**



p electrons drawn toward proton, acting as **base**



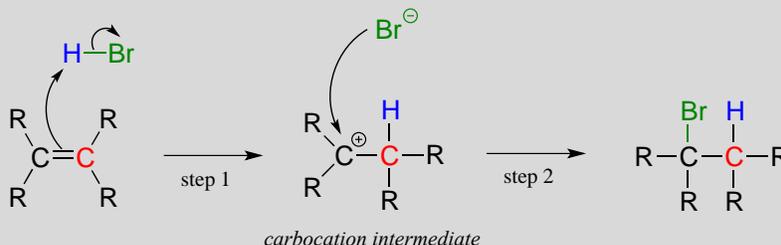
Notice above that, once the π bond breaks and a new σ bond forms, the second carbon that was part of the original π bond becomes a carbocation. Carbocation intermediates play a critical role in this chapter, because a carbocation is a highly reactive species and will quickly attract a pair of electrons. The stability of the carbocation intermediate (recall that we learned about carbocation stability in section 8.5), and the manner in which it accepts a pair of electrons, plays a key role in determining the outcome of the reaction.

Section 14.1: Electrophilic addition

14.1A: Addition of HBr to alkenes

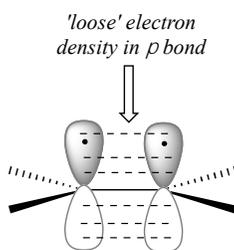
The simplest type of electrophilic reaction to visualize is the addition of a haloacid such as HBr to an isolated alkene. It is not a biological reaction, but nonetheless can serve as a convenient model to introduce some of the most important ideas about electrophilic reactions.

Electrophilic addition of HBr to an alkene:



Step 1 is an acid-base reaction: the π electrons of the alkene act as a base and extract the acidic proton of HBr. This leaves one of the carbons with a new bond to hydrogen, and the other with an incomplete octet and a positive formal charge. In step 2, the nucleophilic bromide anion attacks the electrophilic carbocation to form a new carbon-bromine bond. Overall, the HBr molecule - in the form of a proton and a bromide anion - has been *added* to the double bond.

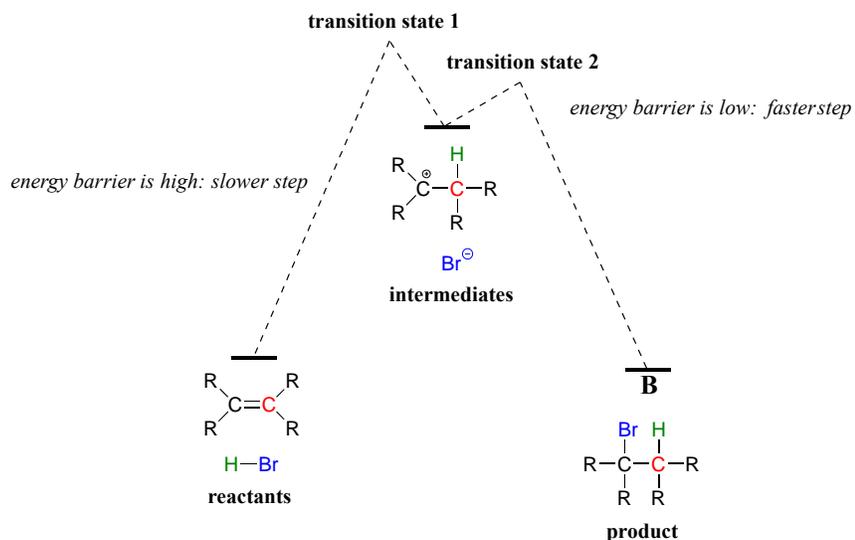
To understand how π -bonded electrons in an alkene could be basic, let's first review the bonding picture for alkenes. Recall (section 2.1C) that the both of the carbons in an alkene group are sp^2 hybridized, meaning that each carbon has three sp^2 hybrid orbitals extending out in the same plane at 180° angles (trigonal planar geometry), and a single, unhybridized p orbital oriented perpendicular to that plane - one lobe above the plane, one lobe below.



The unhybridized p orbitals on the two alkene carbons overlap, in a side-by-side fashion, to form the π bond, which extends above and below the plane formed by the σ bonds. The two electrons shared in this π bond are farther away from the carbon nuclei than the electrons in the carbon-carbon σ bond, and thus are more accessible to the acidic proton. In addition, recall that molecular orbital (MO) theory tells us that π orbitals are higher in energy than σ orbitals (section 2.2). As a consequence, it is easier to break the π bond of an alkene than it is to break the σ bond: the π bond is more reactive.

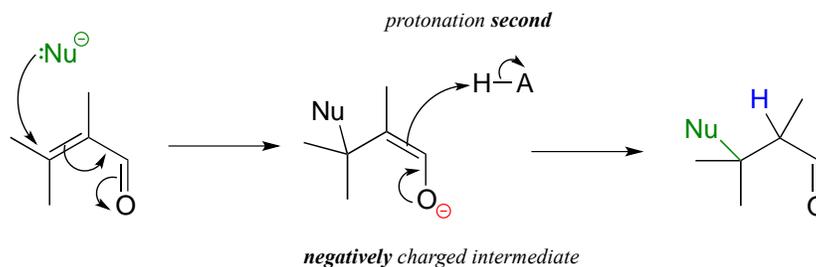
As the HBr molecule approaches the alkene, a new σ bond is formed between one of the alkene carbons and the electron-poor proton from HBr. The carbon, which was sp^2 hybridized when it was part of the alkene, is now sp^3 hybridized. The other alkene carbon is still sp^2 hybridized, but it now bears a positive formal charge because it has only three bonds, and its p orbital is empty. But it won't stay empty for long: a carbocation is a very reactive, unstable intermediate. The bromide ion will rapidly act as a nucleophile, filling the orbital with a pair of electrons, and now with four σ bonds the carbon is sp^3 -hybridized.

The first step in the electrophilic addition reaction is much slower than the second step, because the intermediate carbocation species is higher in energy than either the reactants or the products, and as a result the energy barrier for the first step is also higher than for the second step. The slower first step is the rate-determining step: a change in the rate of the slow step will effect the rate of the overall reaction, while a change in the rate of the fast step will not.

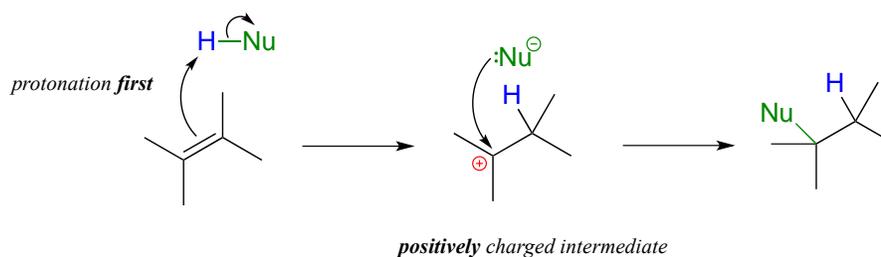


It is important to recognize the inherent difference between an *electrophilic* addition to an alkene and a *conjugate* addition to an alkene in the □□□ position, the latter of which we studied earlier in section 13.4. In both reactions, a proton and a nucleophile add to the double bond of an alkene. In a conjugate addition, the nucleophilic attack takes place first, resulting in a negatively charged intermediate (an enolate). Protonation is the *second* step. Also, of course, the alkene must be conjugated to a carbonyl or imine.

conjugate addition:



electrophilic addition:

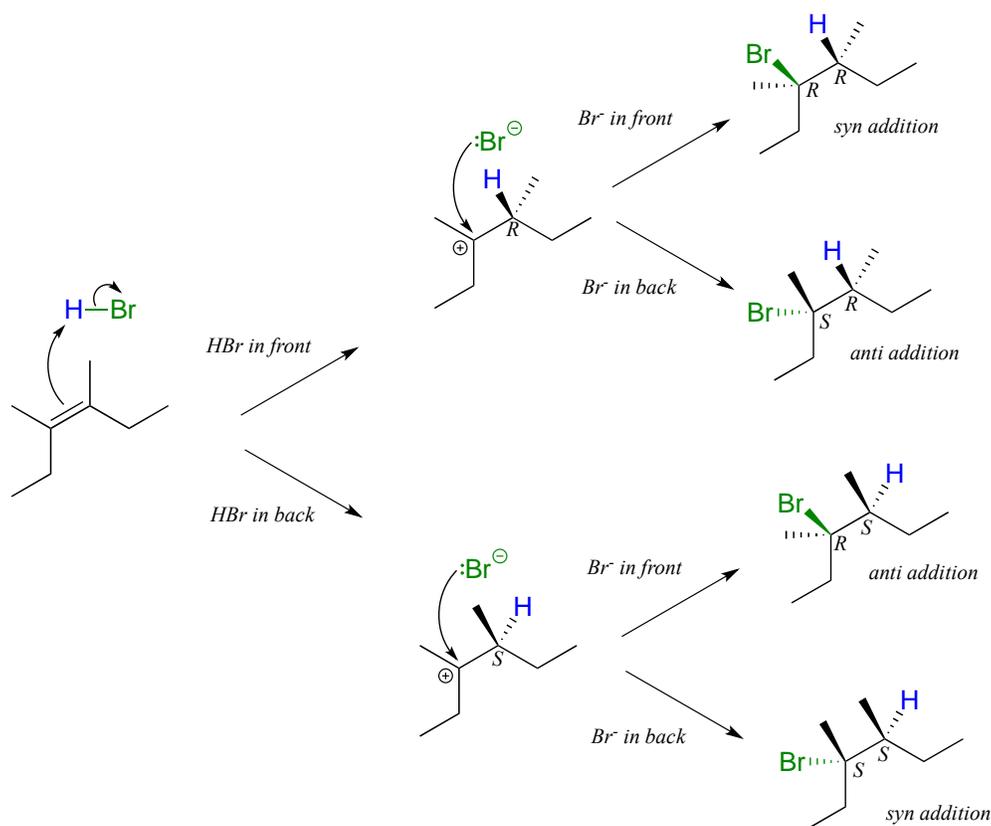


In an electrophilic addition, proton abstraction occurs *first*, generating a positively-charged intermediate. Nucleophilic attack is the second step. No conjugated carbonyl or imine group is required: in fact a nearby carbonyl group would actually slow down a

hypothetical electrophilic addition reaction down because a carbonyl is an electron withdrawing, carbocation-destabilized group.

14.1B: The stereochemistry of electrophilic addition

Depending on the structure of the starting alkene, electrophilic addition has the potential to create two new chiral centers. Addition of HBr to an alkene is *not* stereoselective: the reaction results in racemization at *both* of the alkene carbons. Consider the addition of HBr to *cis*-3,4-dimethyl-3-hexene. The initial proton abstraction step creates a new chiral center, and because the acidic proton could be added to either side of the planar alkene carbon with equal probability, the center could have either *S* or *R* configuration.



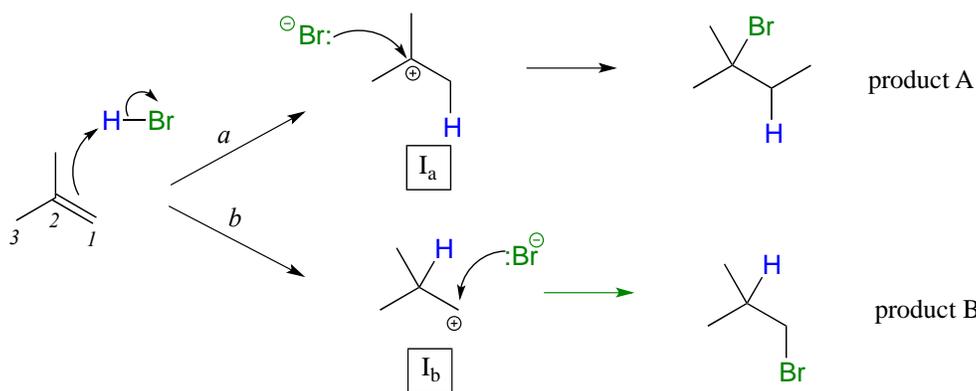
Likewise, in the second step the nucleophilic bromide ion could attack from either side of the planar carbocation, leading to an equal mixture of *S* and *R* configuration at that carbon as well. Therefore, we expect the product mixture to consist of equal amounts of four different stereoisomers.

Exercise 14.1: Predict the product(s) of electrophilic addition of HBr to the following alkenes. Draw all possible stereoisomers that could form, and take care not to draw identical structures twice.

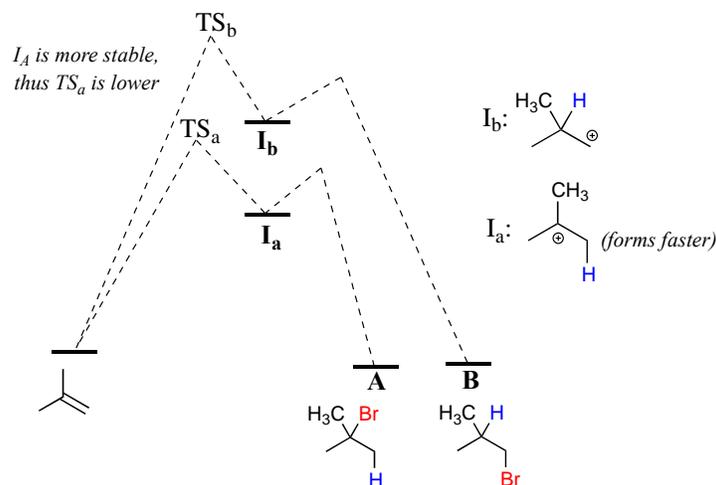
- a) *trans*-2-butene b) *cis*-3-hexene c) cyclopentene

14.1C: The regiochemistry of electrophilic addition

In many cases of electrophilic addition to an alkene, regiochemistry comes into play: the reaction can result in the formation of two different constitutional isomers. Consider the electrophilic addition of HBr to 2-methylpropene:



Note that carbon #1 and carbon #2 in the starting alkene are *not* the same - carbon #2 is bonded to two methyl groups, and carbon #1 to two hydrogen atoms. The initial protonation step could therefore go two different ways, resulting in two different carbocation intermediates. Notice how pathway 'a' gives a tertiary carbocation intermediate (I_a), while pathway 'b' gives a primary carbocation intermediate (I_b). We know from section 8.5 that the tertiary carbocation I_a is lower in energy. Consequently, the transition state $TS(a)$ leading to I_a is lower in energy than $TS(b)$, meaning that I_a forms *faster* than I_b .



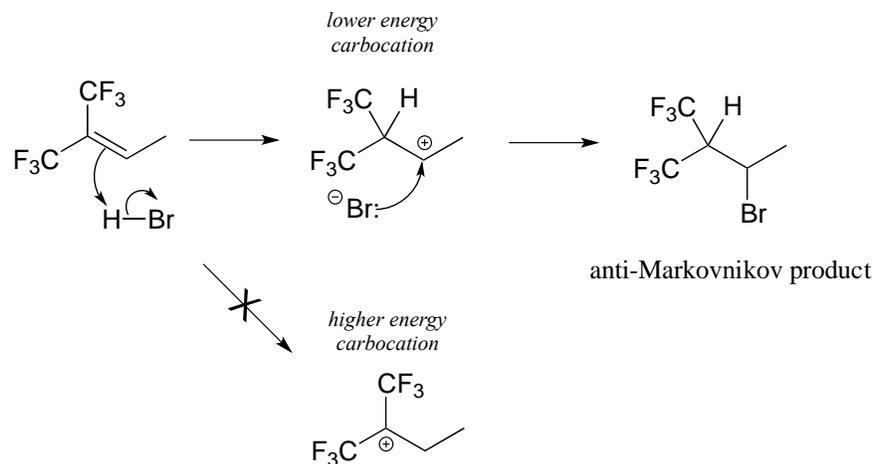
Because the protonation step is the rate determining step for the reaction, tertiary alkyl bromide **A** will form faster than the primary alkyl bromide **B**, and thus **A** will be the predominant product of the reaction. *The electrophilic addition of HBr to 2-methylpropene is regioselective: more than one constitutional isomer can potentially form, but one isomer is favored over the other.* It is generally observed that in electrophilic addition of haloacids to alkenes, the more substituted carbon is the one that ends up bonded to the heteroatom of the acid, while the less substituted carbon is protonated. This 'rule of thumb' is known as **Markovnikov's rule**, after the Russian chemist Vladimir Markovnikov who proposed it in 1869.

While it is useful in many cases, Markovnikov's rule does not apply to all electrophilic addition reactions. It is better to use a more general principle:

The regioselectivity of electrophilic addition

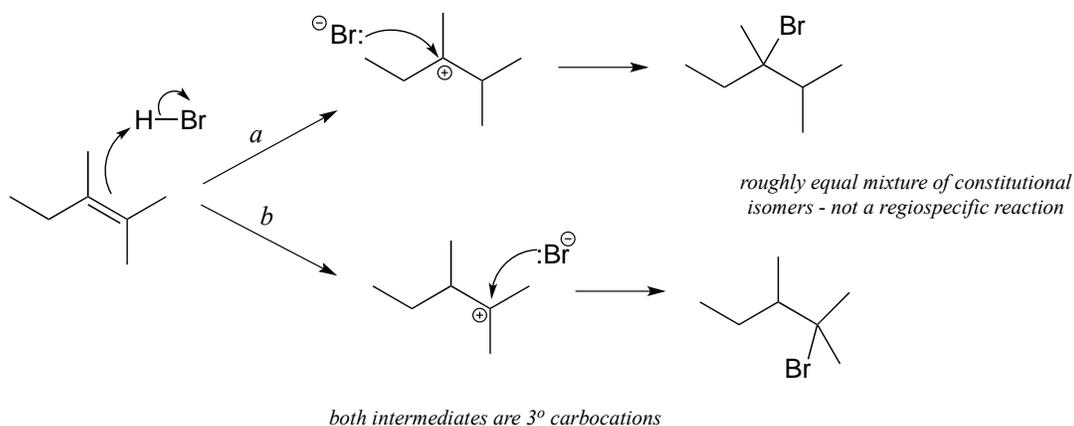
When an asymmetrical alkene undergoes electrophilic addition, the product that predominates is the one that results from the more stable of the two possible carbocation intermediates.

How is this different from Markovnikov's original rule? Consider the following hypothetical reaction, in which the starting alkene incorporates two trifluoromethyl substituents:



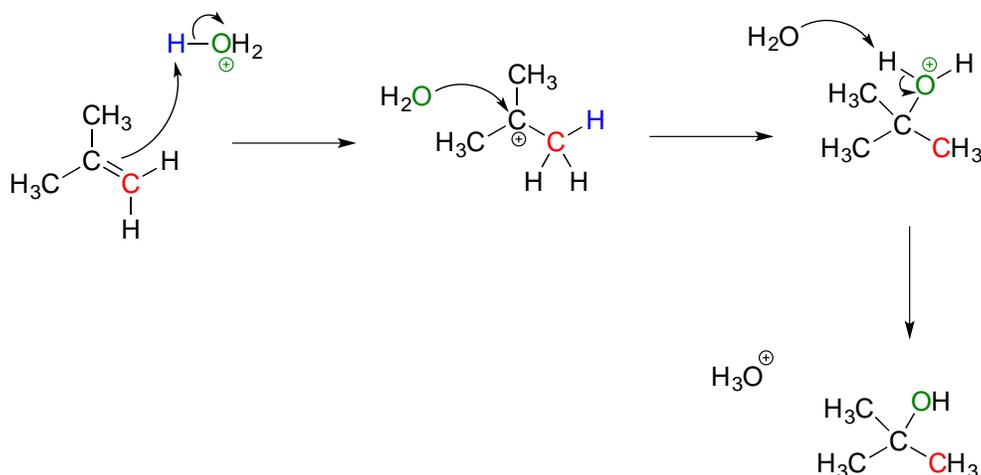
Now when HBr is added, it is the *less* substituted carbocation that forms faster in the rate-determining protonation step, because in this intermediate the carbon bearing the positive charge is located further away from the electron-withdrawing, cation-destabilizing fluorines. As a result, the predominant product is the secondary rather than the tertiary bromoalkane. This is referred to as an **anti-Markovnikov** addition product, because it 'breaks' Markovnikov's rule.

If the two possible carbocation intermediates in an electrophilic addition reaction are of similar stability, the product will be a mixture of constitutional isomers.

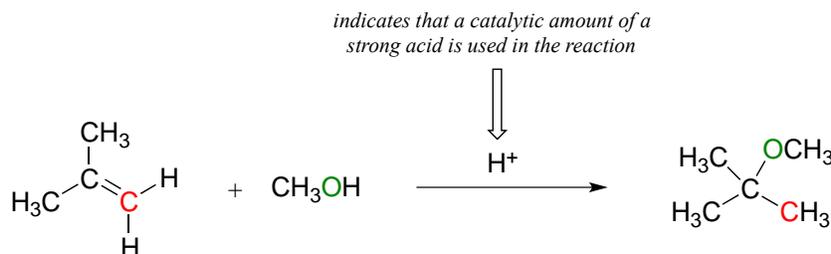


14.1D: Electrophilic addition of water and alcohol

The (non-biochemical) addition of water to an alkene is very similar mechanistically to the addition of a haloacid such as HBr or HCl, and the same stereochemical and regiochemical principles apply. A catalytic amount of a strong acid such as phosphoric or sulfuric acid is required, so that the acidic species in solution is actually H_3O^+ . Note that H_3O^+ is regenerated in the course of the reaction.



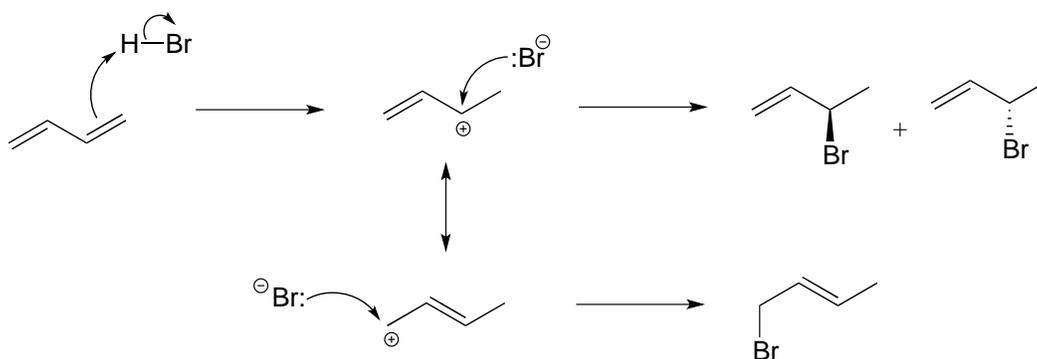
If an alkene is treated with methanol and a catalytic amount of strong acid, the result is an ether:



Exercise 14.2: Draw a mechanism for the ether-forming reaction above.

14.1E: Addition to conjugated alkenes

Electrophilic addition to conjugated alkenes presents additional regiochemical possibilities, due to resonance delocalization of the allylic carbocation intermediate. Addition of one molar equivalent of HBr to 1,3-butadiene, for example, leads to a mixture of three products, two of which are a pair of enantiomers due to the creation of a chiral center at carbon #2.



Exercise 14.3: Explain why 4-bromo-1-butene is *not* a significant product of the reaction above.

Exercise 14.4: Predict the major product(s) of the following reactions. Draw all possible stereoisomers, and take care not to draw the same structure twice.

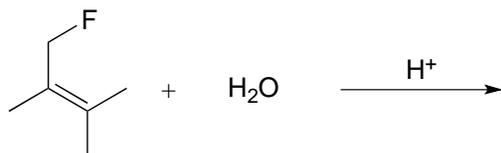
a)



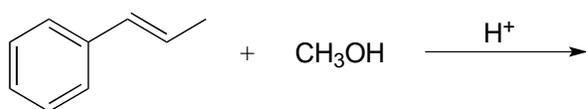
b)



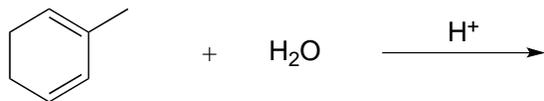
c)



d) *Hint* - are the double bonds in an aromatic ring likely to undergo electrophilic addition?

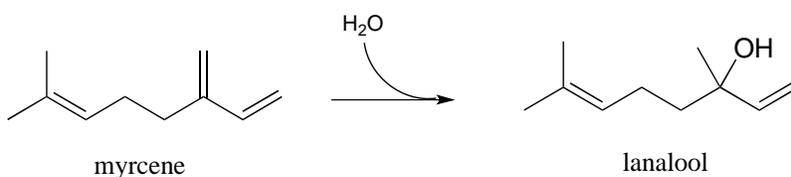


e)



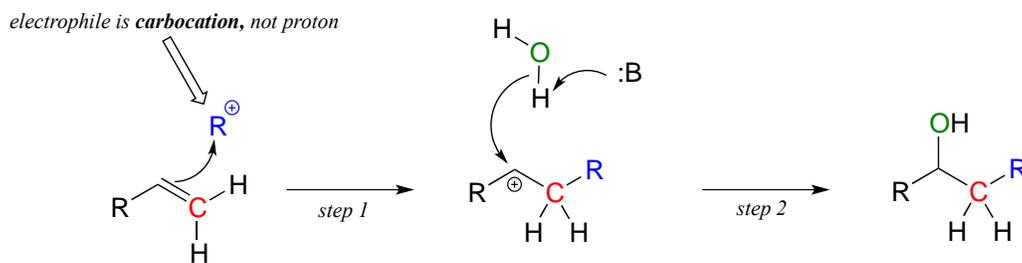
14.1F: Biochemical electrophilic addition reactions

Myrcene is an isoprenoid compound synthesized by many different kinds of plants and used in the preparation of perfumes. Recently an enzymatic pathway for the degradation of myrcene has been identified in bacteria (*J. Biol. Chem* **2010**, 285, 30436). The first step of this pathway is electrophilic addition of water to a conjugated alkene system.

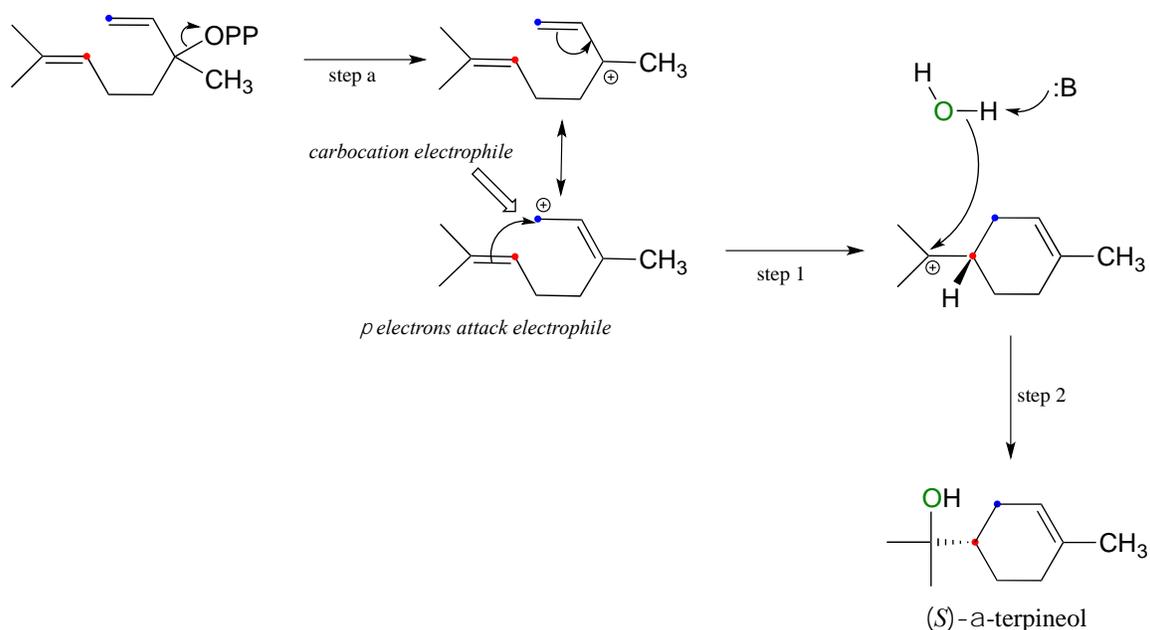


Exercise 14.5: Draw a mechanism for the above reaction, showing two resonance contributors of the carbocation intermediate. How would you characterize the intermediate?

Although the hydration of myrcene above looks very familiar, many enzyme-catalyzed electrophilic addition reactions differ from what we have seen so far, in that the electron-poor species attacked by the π -bonded electrons in the initial step is a *carbocation* rather than an acidic proton:



□ *lpha*-terpineol, a major component in the sap of pine trees, is formed in an electrophilic addition reaction. The first thing that happens (which we will refer to below as 'step a', in order to keep the step numbering consistent with the addition mechanisms we have seen so far) is departure of a pyrophosphate leaving group, forming an allylic carbocation electrophile.



The actual electrophilic addition stage of the reaction begins with step 1, as the π electrons of an alkene are drawn toward one of the two carbons that share the positive charge, effectively closing a six-membered ring. A water molecule then attacks the second carbocation intermediate (step 2), which completes the addition process.

Notice something important about the regiochemical course of the reaction: step 1 results in the formation of a six-membered ring and a tertiary carbocation. As we have stressed before, biochemical reactions tend to follow energetically favorable mechanistic pathways.

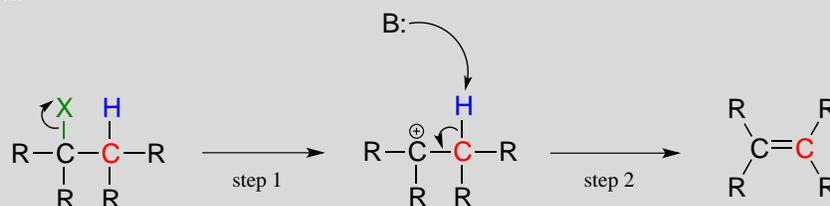
Exercise 14.6: An alternate regiochemical course to step 1 shown above could result in a seven-membered ring and a secondary carbocation, a much less energetically favorable intermediate in terms of both carbocation stability and ring size. Draw a mechanism for this hypothetical alternate reaction, and show the product that would result after the addition of water in a hypothetical 'step 2'.

Section 14.2: Elimination by the E1 mechanism

14.2A: E1 elimination - an overview

The reverse of electrophilic addition is called **E1 elimination**. We will begin by looking at some non-biochemical E1 reactions, as the E1 mechanism is actually somewhat unusual in biochemical pathways.

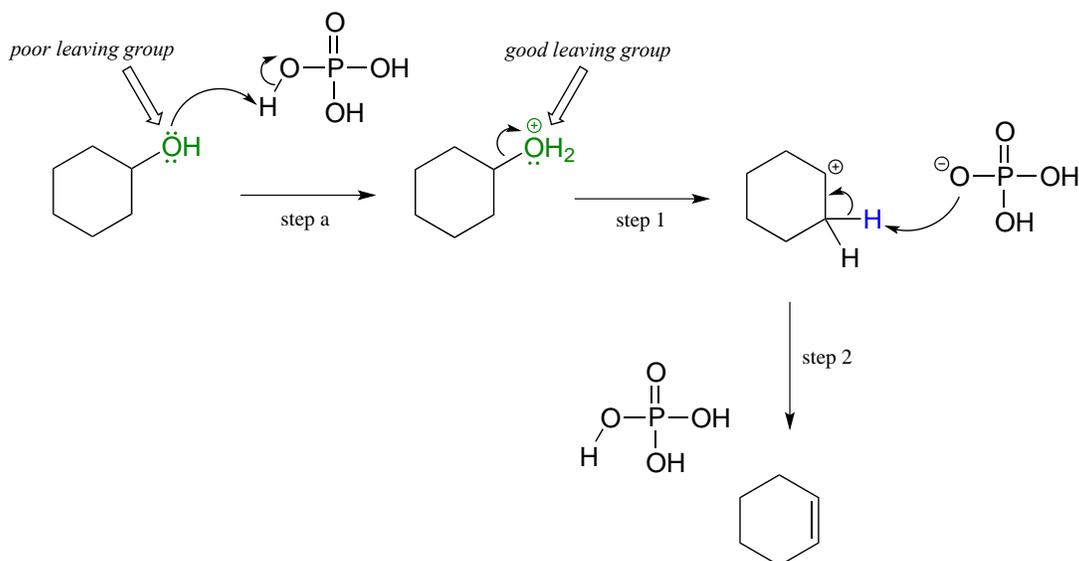
E1 elimination:



An E1 elimination begins with the departure of a leaving group (designated 'X' in the general figure above) and formation of a carbocation intermediate (step 1). Abstraction of a proton from an adjacent carbon (step 2) sends two electrons down to fill the empty p orbital of the carbocation, forming a new π bond. The base in this step may be the leaving group, or another basic species in solution.

E1 elimination does not occur when the leaving group is bonded to a primary carbon, unless the carbon is in the allylic or benzylic position. Recall that a primary carbocation, unless stabilized by resonance, is highly unstable and an unlikely reaction intermediate.

E1 eliminations can occur at secondary carbons, however. If cyclohexanol is heated with a catalytic amount of phosphoric acid, elimination of water (dehydration) results in cyclohexene as the product. The role of the phosphoric acid is to protonate the alcohol ('step a' below), making it a viable leaving group.



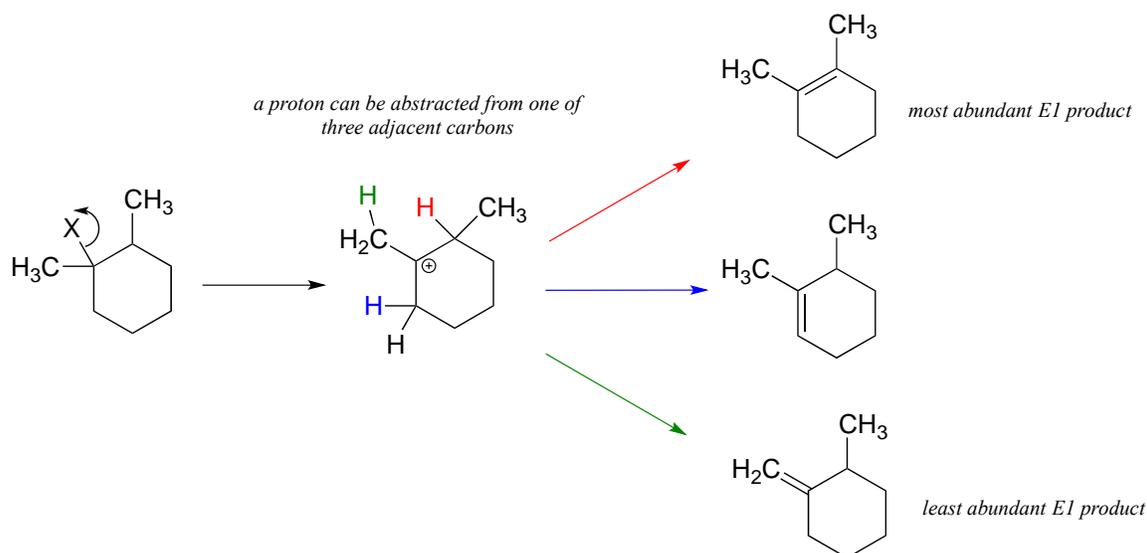
The reaction is reversible, but if cyclohexene is distilled away from the reaction mixture as it forms, the equilibrium can be driven towards product (you may want to review Le Chatelier's principle in your General Chemistry textbook). Separation of cyclohexene

(boiling point 83 °C) from cyclohexanol (boiling point 161 °C) is simple because of the large difference in boiling points between the two liquids.

Exercise 14.7: When the laboratory reaction described above is run to completion, a viscous 'goop' is usually left over in the distillation flask, which hardens upon cooling. Draw a mechanism showing how this 'goop' might form, and explain why it hardens upon cooling.

14.2B: Regiochemistry of E1 elimination

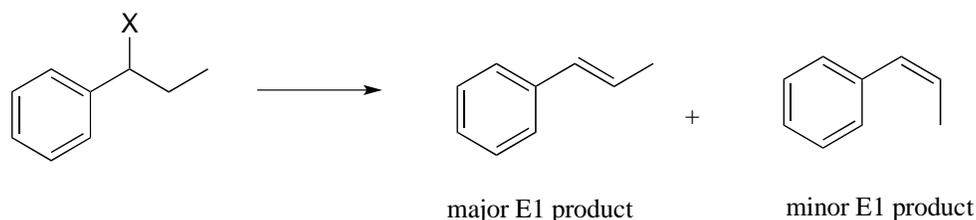
Nonenzymatic E1 reactions can often result in a mixture of more than one alkene product. Elimination of 'HX' from the following starting compound, for example, could yield three different possible alkene products.



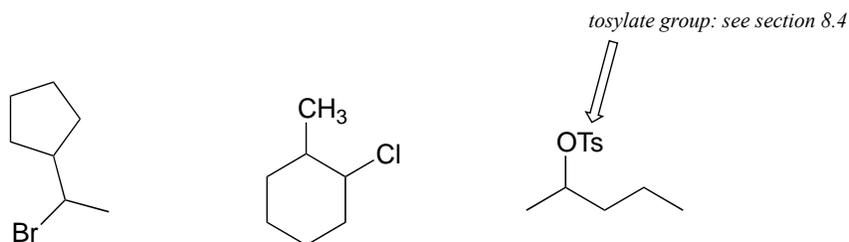
Notice in the figure above that the three possible E1 products do not form in equal abundance. *The most abundant alkene product is that which is most substituted:* in other words, the alkene in which the two sp^2 carbons are bonded to the fewest hydrogen atoms. In this case, the most substituted alkene has zero hydrogen substituents. The least substituted - and least abundant - alkene product has two hydrogen substituents, while the middle alkene has one hydrogen substituent. This trend is observed generally with nonenzymatic E1 elimination reactions, and is known as **Zaitsev's rule** after the Russian chemist Alexander Zaitsev.

14.2C: Stereochemistry of E1 elimination

Nonenzymatic E1 reactions can also result in both *cis* and *trans* alkenes. Keeping in mind that in general *trans* alkenes are more stable than *cis* alkenes, we can predict that *trans* alkenes will predominate in the product mixture.



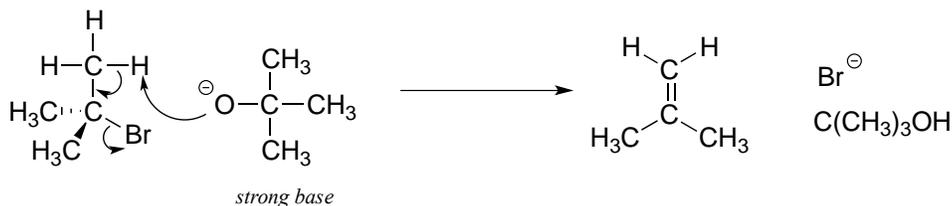
Exercise 14.8: Draw the structures of all possible E1 products starting with the compounds below, and rank them in order of highest to lowest abundance.



14.2D: The E2 elimination mechanism

When a strong base is combined with an alkyl halide (or alkyl tosylate/mesylate), elimination generally occurs by the **E2 pathway**, in which proton abstraction and loss of the leaving group occur *simultaneously*, without an intervening carbocation intermediate:

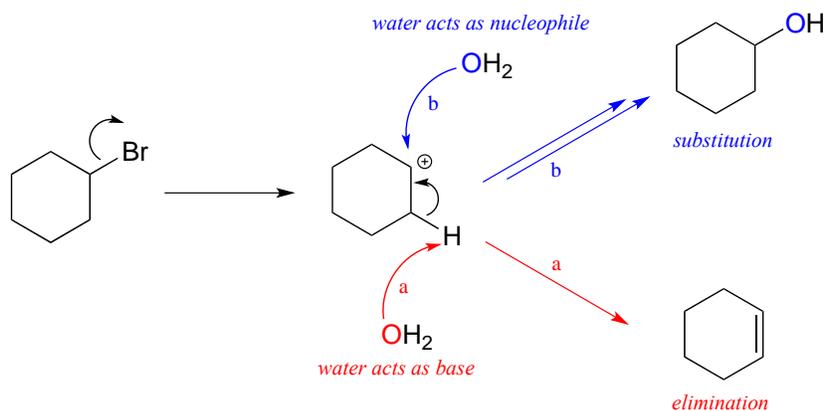
bond-breaking and bond-forming occurs simultaneously, no carbocation intermediate



Just like in the S_N2 mechanism, the '2' in the E2 designation refers to the idea that the rate-determining (and only) step of the reaction is a collision between the two reacting molecules, in this case bromocyclohexane and methoxide ion.

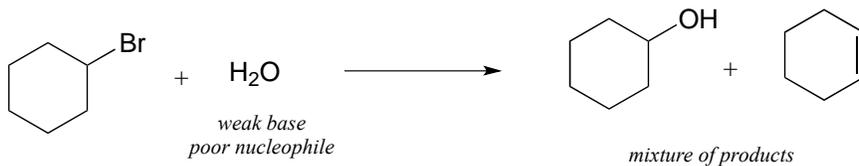
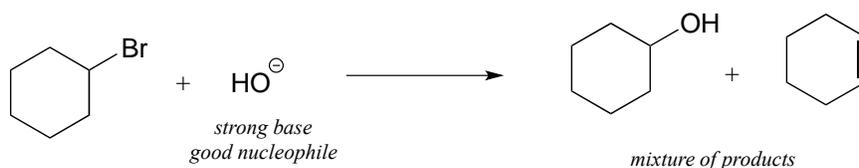
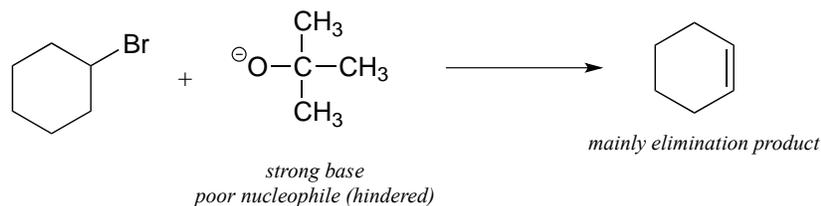
14.2E: Competition between elimination and substitution

Consider a reaction between water and bromocyclohexane. Based on what we have just learned, a likely product would be the alkene formed from an E1 elimination reaction (pathway (a) in red below).

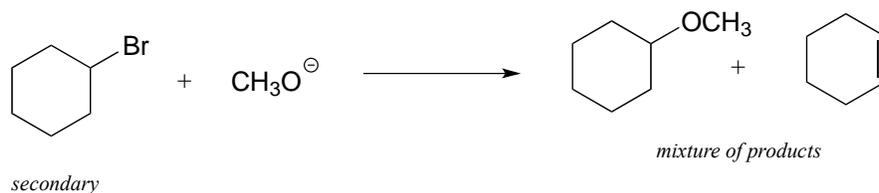


However, the reaction could take another course: what if the water molecule, instead of acting as a base, were to act as a nucleophile (pathway (b) in blue)? This should look familiar - it is simply an S_N1 reaction (section 8.1B). In fact, the reaction would result in a mixture of elimination (E1) and substitution (S_N1) products. This is a common theme: elimination and substitution often compete with each other.

When both elimination and substitution products are possible, however, we can often predict which reaction will predominate. In general, *strong bases and hindered carbons favor elimination, while powerful nucleophiles and unhindered carbons favor substitution.*



In addition, primary alkyl halides are much more likely to undergo substitution than elimination, even when the nucleophile is also a strong base, because the electrophilic carbon is unhindered and accessible to the nucleophile. Recall that the Williamson ether synthesis (section 8.9A) is an efficient laboratory $\text{S}_{\text{N}}2$ reaction between a primary (or methyl) alkyl halide and an alkoxide. If a secondary alkyl halide is used, a substantial amount of elimination product will form (the electrophilic carbon is more hindered, and the alkoxide will act as a base as well as a nucleophile).



While competition between substitution and elimination pathways is an issue for chemists running reactions in the lab, the same cannot be said of biochemical reactions, as the architecture active site of enzymes have evolved to ensure the formation of only one product.

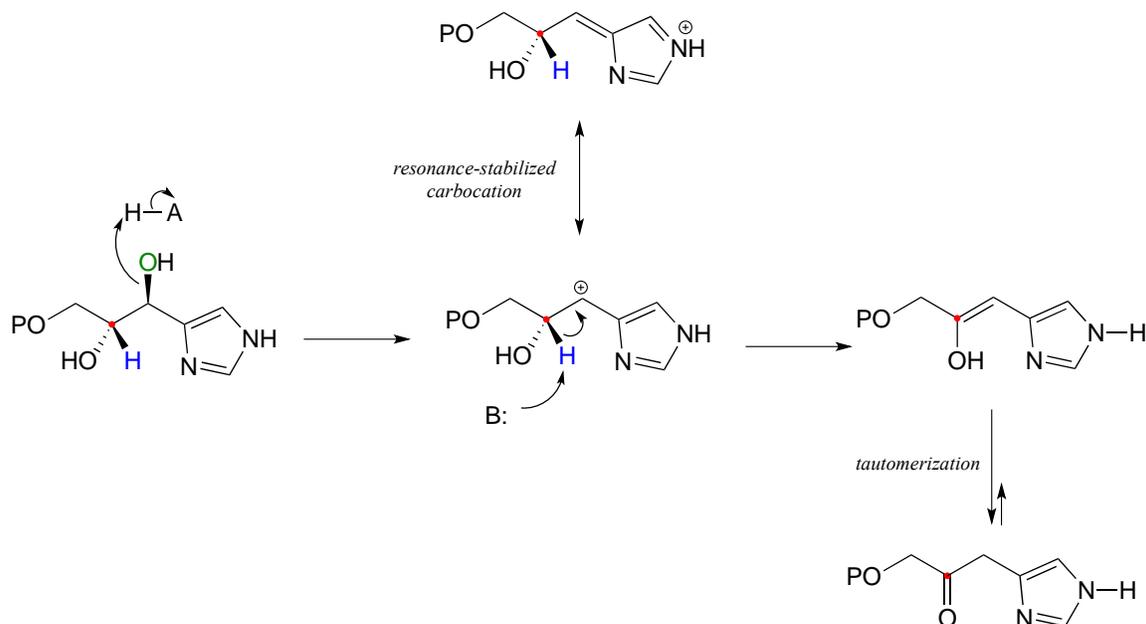
Exercise 14.9: Predict the *major* organic product(s) of the following reactions. If the reaction is expected to result in a mixture of elimination and substitution product, show both.

- bromocyclopentane plus ethoxide
- 1-chlorohexane plus CH_3S^-
- 2-iodo-2-methylpentane plus hydroxide

14.2F: Biochemical E1 elimination reactions

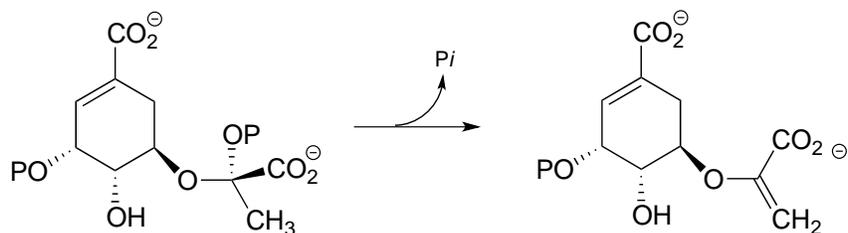
Looking through metabolic pathways in a biochemistry textbook, you'll see that almost all elimination reactions appear to be of the E1cb type, occurring on carbons in the α position relative to a carbonyl or imine. A relatively small number of elimination steps, however, take place away from the electron-withdrawing influence of a carbonyl or imine, and these are of the carbocation-intermediate, E1 type. The E2 mechanism is very rare in biochemical pathways.

A reaction in the histidine biosynthetic pathway (EC 4.2.1.19) provides an example of a biological E1 dehydration step:



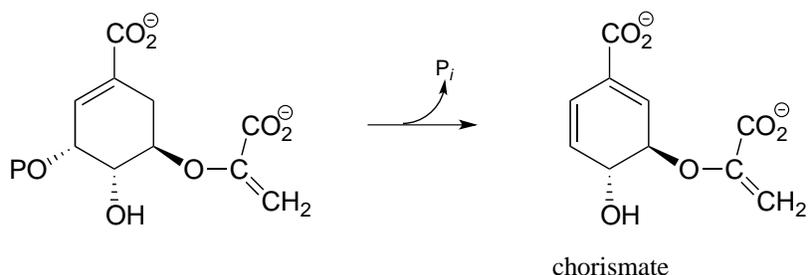
Notice that an E1cb mechanism is not possible here - there is no adjacent carbonyl or imine and thus no possibility for a stabilized anionic intermediate. Instead, the first step is loss of water to form a resonance-stabilized carbocation intermediate. Deprotonation completes the E1 phase of the reaction to form an enol, which rapidly tautomerizes to a ketone.

Another example of a biological E1 reaction is found in the biosynthesis pathway for aromatic amino acids (EC 2.5.1.19):

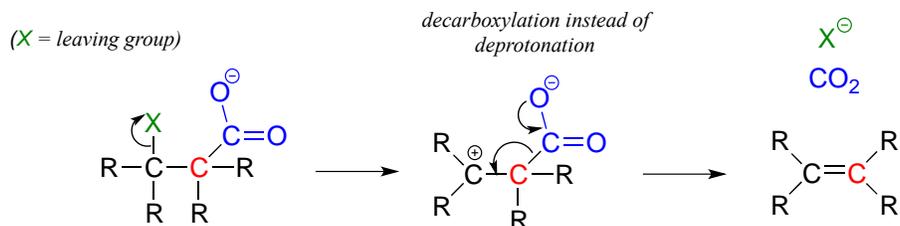


Exercise 14.10: Draw a complete mechanism for the reaction above. Show how the carbocation intermediate is stabilized by resonance.

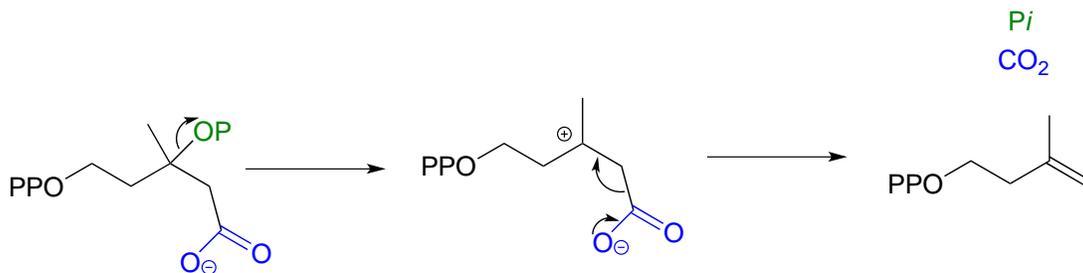
Exercise 14.11: Another step (EC 4.2.3.5) in the aromatic acid biosynthesis pathway could be referred to as a *conjugated* E1 elimination of phosphate, the mechanistic reverse of electrophilic addition to a conjugated diene (section 14.1E). Draw a complete mechanism for this reaction, showing two resonance contributors of the carbocation intermediate.



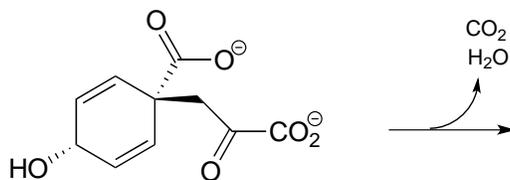
In section 13.3, we saw some Claisen condensation reactions in which the usual proton-abstractor step was replaced by decarboxylation. A similar thing can happen with E1 eliminations:



Isopentenyl diphosphate, the 'building block' for all isoprenoid compounds, is a product of this type of hybrid decarboxylation / elimination reaction (EC 4.1.1.33).



Exercise 14.12: A *conjugated* decarboxylation/E1 elimination reaction (EC 4.2.1.51) occurs in the phenylalanine biosynthesis pathway.

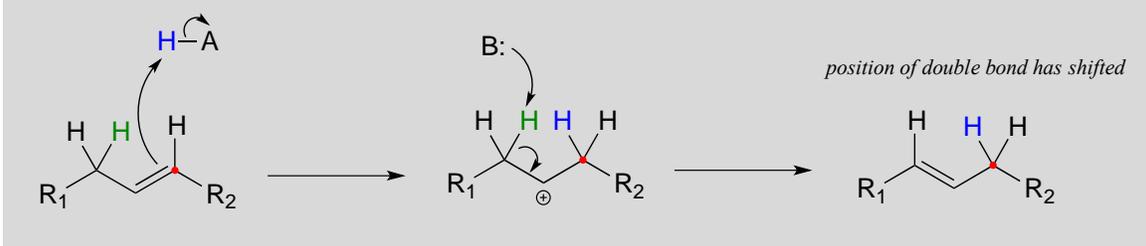


- Predict the product, and draw a mechanism.
- What two main factors contribute to the 'driving force' for this reaction?

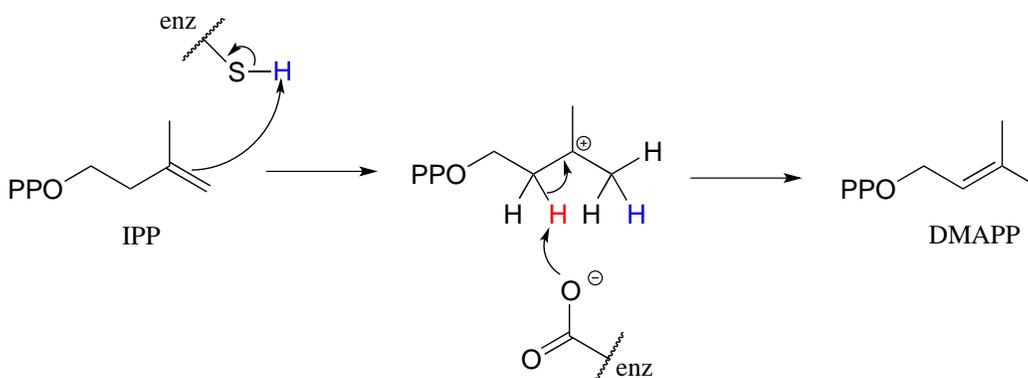
Section 14.3: Electrophilic alkene isomeration

Electrophilic reactions in biochemistry are not limited to *addition* to alkene double bonds. The position of a double bond in an alkene can also be shifted through an electrophilic, carbocation-intermediate reaction. An electrophilic alkene *isomerization* occurs when an initial π bond protonation event (step 1 below) is followed by deprotonation of an adjacent carbon to re-form the π bond in a different location.

Electrophilic isomerization mechanism:



In a key early step in the biosynthesis of isoprenoid compounds, isopentenyl diphosphate (IPP), the isoprenoid 'building block' molecule, is isomerized to dimethylallyl diphosphate (DMAPP) (EC 5.3.3.2).

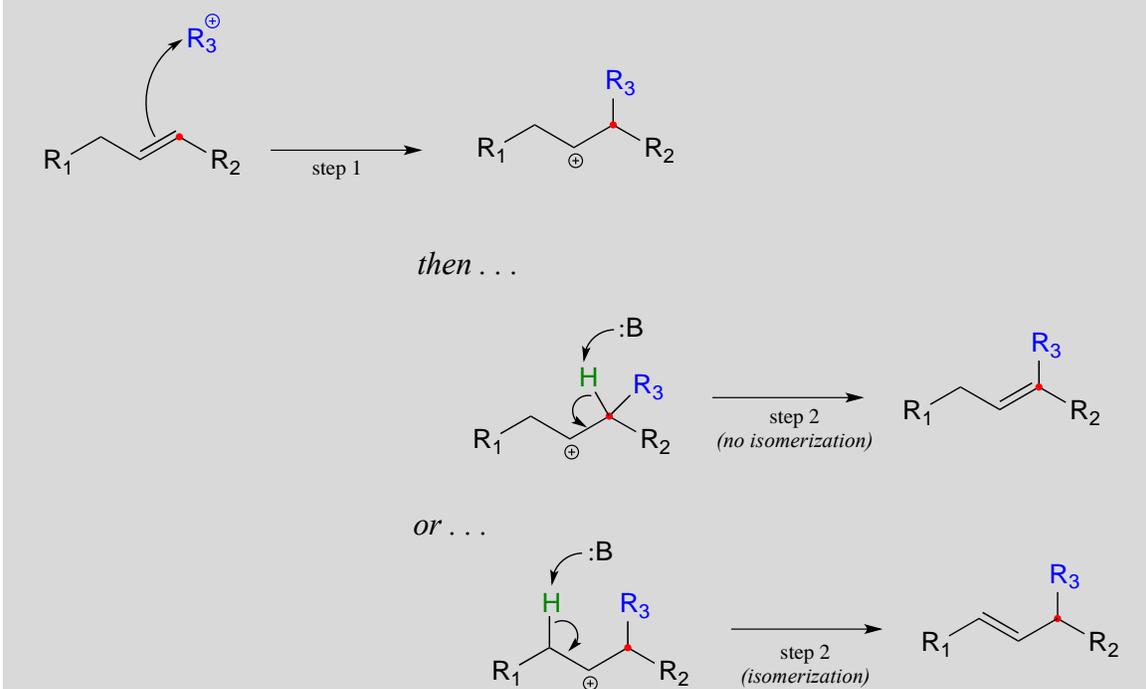


In the first step, the π bond between carbon #3 and carbon #4 is protonated by a cysteine residue in the active site. X-ray crystallography studies on the isomerase enzyme (*EMBO J.* **2001**, *20*, 1530) show that the carbocation intermediate is bound in a very deep, hydrophobic active site cavity that seals out any water molecules that could potentially attack the carbocation to form an undesired alcohol product. Instead, a basic glutamate residue is positioned in the active site to abstract a proton from carbon #2 (step 2), serving to reestablish the double bond in a new position between carbons #2 and #3.

Section 14.4: Electrophilic substitution

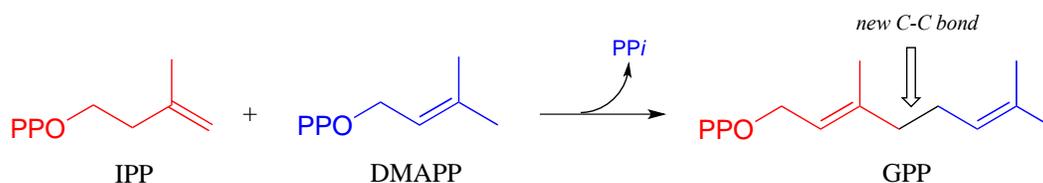
We have already been introduced to electrophilic addition and electrophilic isomerization - now, let's move to the third variation on the electrophilic theme, that of electrophilic substitution. In an electrophilic substitution reaction, a pair of π -bonded electrons first attacks an electrophile - usually a carbocation species - and a proton is then abstracted from an adjacent carbon to reestablish the double bond, either in the original position or with isomerization.

Electrophilic substitution mechanism:

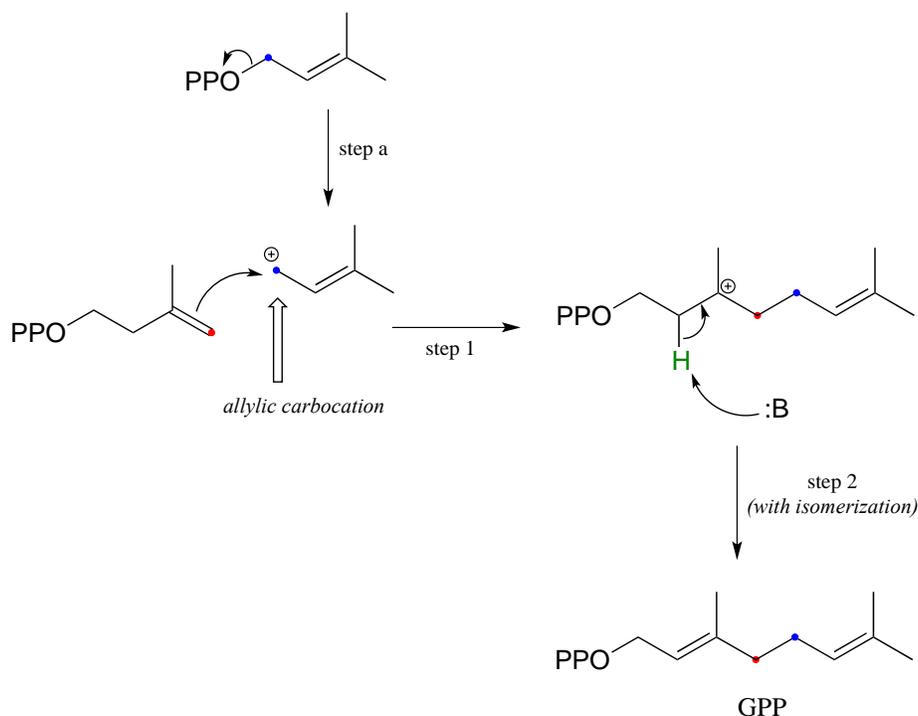


14.4A: Electrophilic substitution reactions in isoprenoid biosynthesis

Electrophilic substitution steps are very important in the biosynthetic pathways of isoprenoid compounds. In an early, chain-elongating reaction (EC 2.5.1.1) of the pathways of many isoprenoids, building blocks IPP and DMAPP combine to form a 10-carbon isoprenoid product called geranyl diphosphate (GPP):



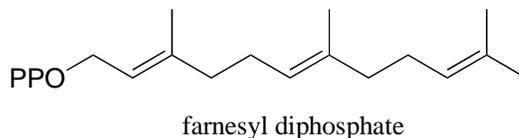
In a preliminary step (step a below), the diphosphate group on DMAPP departs to form an allylic carbocation.



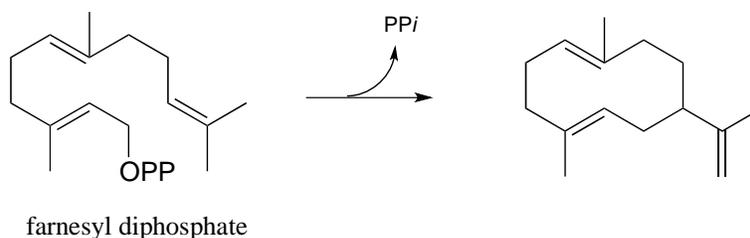
In step 1, the π electrons in IPP then attack the electrophilic carbocation from step a, resulting in a new carbon-carbon bond and a tertiary carbocation intermediate. Proton abstraction (step 2) leads to re-establishment of a double bond one carbon over from where it started out in IPP.

Exercise 14.13: DMAPP is much more prone to spontaneous hydrolysis than IPP when they are dissolved in water. Explain why.

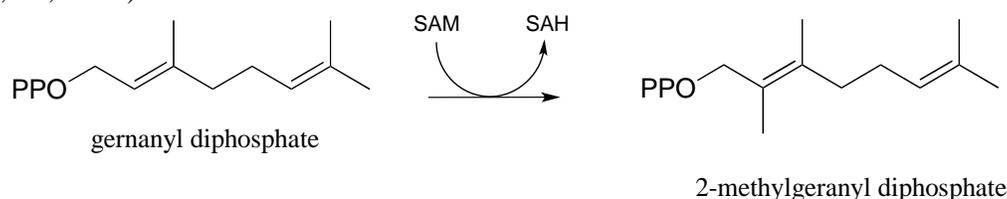
Exercise 14.14: Farnesyl diphosphate (FPP) is synthesized by adding another five-carbon building block to geranyl diphosphate. What is this building block - IPP or DMAPP? Draw a mechanism for the formation of FPP.



Exercise 14.15: Propose a likely mechanism for the following transformation, which is the first stage in a somewhat complex reaction in the synthesis of an isoprenoid compound in plants. (*Science* **1997**, 277, 1815)



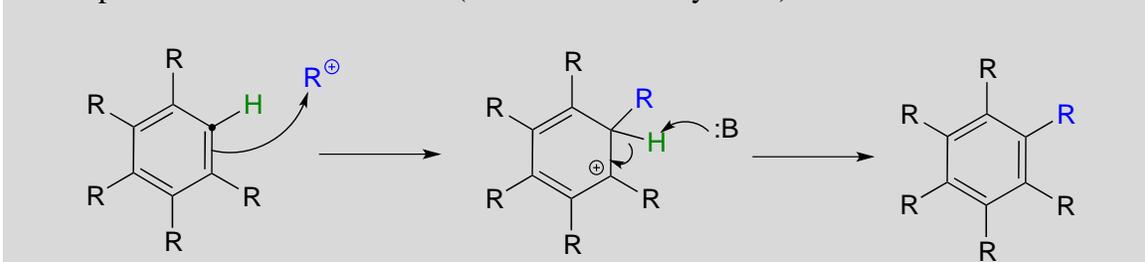
Exercise 14.16: The electrophilic carbon in an electrophilic substitution reaction is often a carbocation, but it can also be the methyl group on S-adenosylmethionine (SAM - see section 8.8A). Propose a likely mechanism for this methylation reaction. (*Biochemistry* **2012**, 51, 3003)



14.4B: Electrophilic aromatic substitution

Until now, we have been focusing mostly on electrophilic reactions of alkenes. Recall from section 2.2C that π bonds in aromatic rings are substantially less reactive than those in alkenes. Aromatic systems, however, do in fact undergo electrophilic substitution reactions given a powerful electrophile such as a carbocation, and if the carbocation intermediate that forms can be sufficiently stabilized.

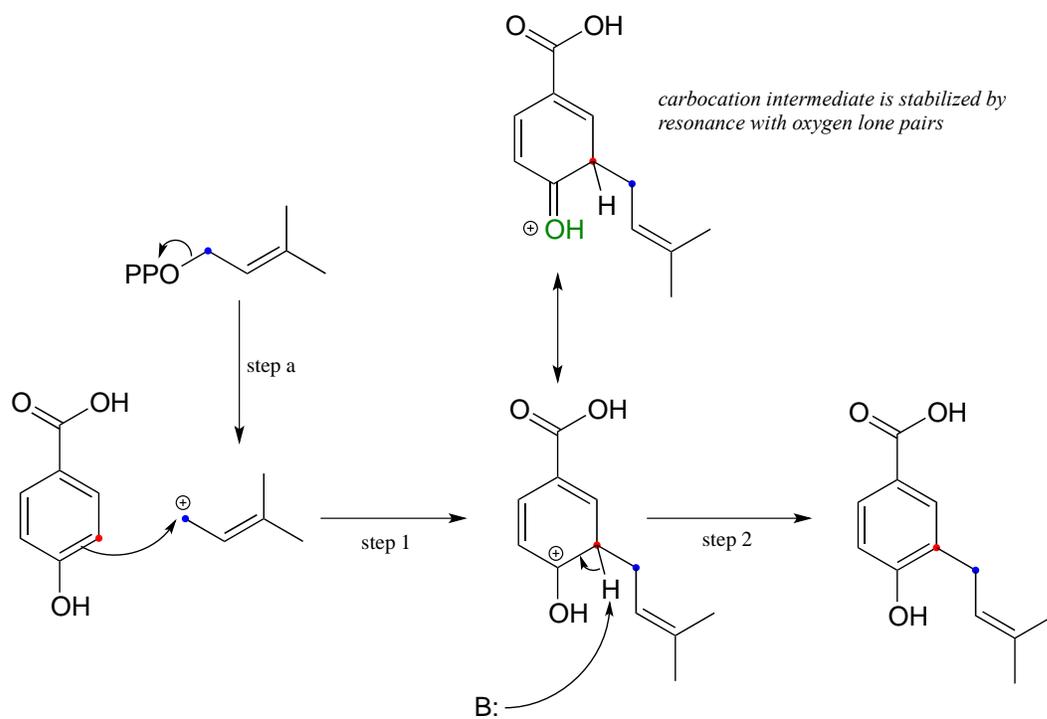
Electrophilic aromatic substitution (Friedel-Crafts alkylation) mechanism



Organic chemists often refer to electrophilic aromatic substitution reactions with carbocation electrophiles as **Friedel-Crafts alkylation** reactions.

Exercise 14.17: Aromatic rings generally do not undergo electrophilic *addition* reactions. Why not?

The Friedel-Crafts reaction below is part of the biosynthesis of vitamin K and related biomolecules.



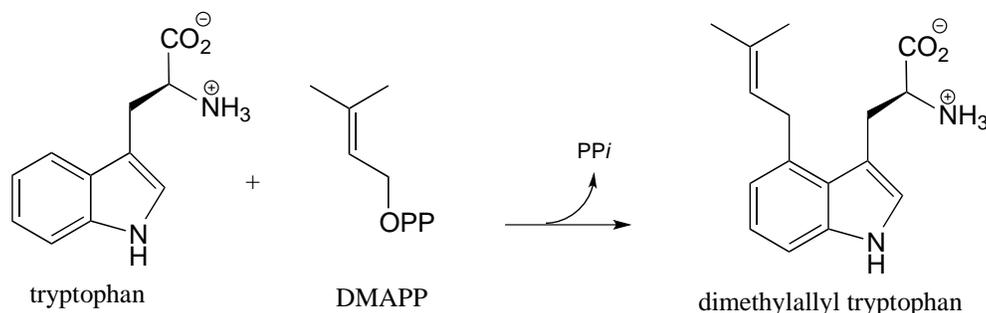
Loss of diphosphate creates a powerful carbocation electrophile (step a) which attracts the π electrons of the aromatic ring to form a carbocation intermediate with a new carbon-carbon bond (step 1). Substitution is completed by proton abstraction (step 2) which re-establishes the aromatic sextet.

An important point must be made here: because aromatic π bonds are substantially less reactive than alkene π bonds, the electrophile must be VERY electrophilic - usually a carbocation. In addition, the carbocation intermediate that results from attack by aromatic π electrons is generally stabilized by resonance with lone pair electrons on a nearby oxygen or nitrogen (look at the resonance forms of the positively-charged intermediate that forms as the result of step 1 in the above figure).

Remember that stabilizing the intermediate formed in a rate-limiting step has the effect of lowering the activation energy for the step, and thus accelerating the reaction.

Organic chemists use the term **ring activation** to refer to the rate-accelerating effect of electron-donating heteroatoms in electrophilic aromatic substitution reactions. Aromatic rings lacking any activating oxygen or nitrogen atoms are less reactive towards electrophilic substitution.

An example of the ring-activating effect of the nitrogen atom on an aromatic ring can be found in the following Friedel-Crafts reaction (EC 2.5.1.34), which should be familiar from the introduction to this chapter:



Recall that this is a key early step in the biosynthetic pathway for the ergot alkaloids which are hypothesized to have been the root cause of the 'bewitchment' of several young girls in 17th century Salem, Massachusetts.

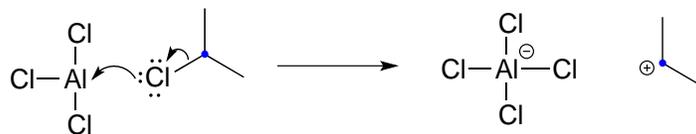
Exercise 14.18: Draw a likely mechanism for the biosynthesis of dimethylallyl tryptophan, including a resonance structure showing how the carbocation intermediate in the rate determining step is stabilized by lone pair electrons on the ring nitrogen (in other words, show how the nitrogen serves to *activate* the ring).

Friedel-Crafts reactions, in addition to being important biochemical transformations, are commonly carried out in the laboratory. It is instructive to consider a few examples to see how the same principles of structure and reactivity apply to both biochemical and laboratory reactions.

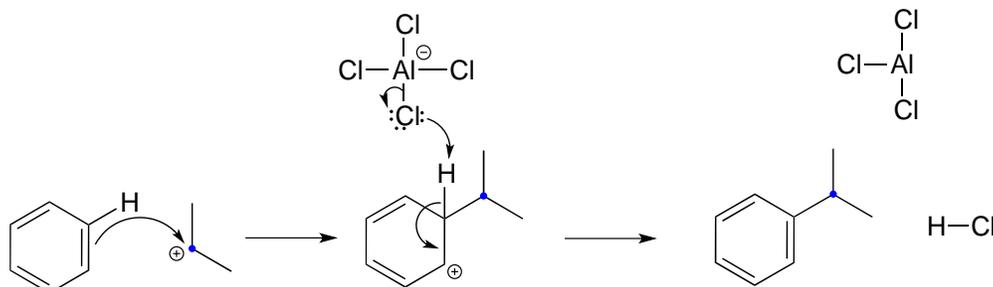
Below is an example of a laboratory Friedel-Crafts alkylation reaction:



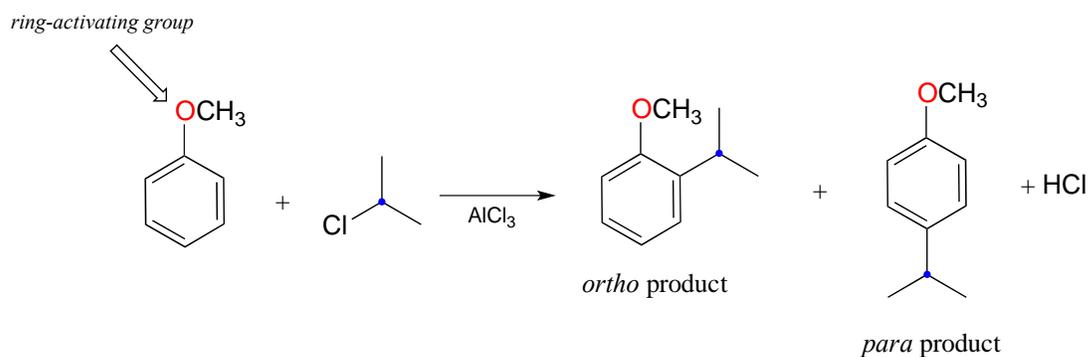
Recall that a powerful electrophile - such as a carbocation - is required for an electrophilic aromatic substitution to occur. The 2-chloropropane reactant is electrophilic, but not electrophilic enough to react with benzene. Here's where the aluminum trichloride catalyst comes in: it reacts as a Lewis acid with the alkyl chloride to generate a secondary carbocation:



The carbocation thus generated is sufficiently electrophilic to react with the aromatic π electrons, in a manner that should be familiar from the biochemical examples discussed above:



You may have noticed, however, that one element from the biochemical Friedel-Crafts reactions is missing here: there is no activating group to stabilize the ring carbocation intermediate. Indeed, the presence of an activating group - for example, the oxygen atom of a methoxy substituent - greatly increases the rate of a Friedel-Crafts alkylation.



Note in the example shown above that two products are formed: one is an *ortho*-disubstituted benzene and one is *para*-disubstituted. Note also that no *meta*-disubstituted product is formed. This phenomenon is referred to as the ***ortho-para* directing effect**, and you are led towards an explanation in the exercise below.

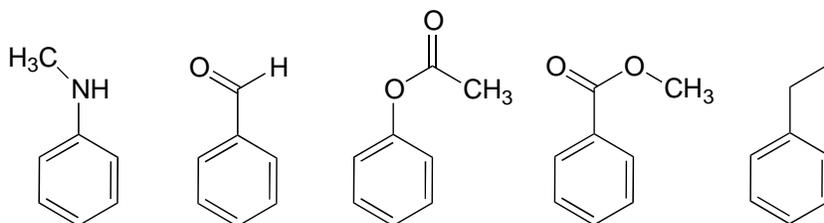
Exercise 14.19:

a) Draw the lowest-energy resonance contributors of the carbocation intermediates leading to formation of the *ortho* and *para* products in the reaction above. Use resonance structures to illustrate how the methoxy substituent is a ring-activating group.

b) Draw the *hypothetical* carbocation intermediate in a reaction leading to formation of a *meta*-disubstituted product. Is this carbocation stabilized by the methoxy oxygen? Can you see why no *meta* product forms?

Exercise 14.20:

a) Just as there are ring-activating groups in electrophilic aromatic substitutions, there are also ring-*de*activating groups. For each of the substituted benzene reactants below, draw the carbocation intermediate leading to the *ortho* substitution product and decide whether the substituent is ring-activating or ring-deactivating in a Friedel-Crafts reaction with 2-chloropropane and AlCl_3 (in other words, which compounds would react *faster* than benzene, and which would react *slower*?) Explain how the ring-deactivating effect works.

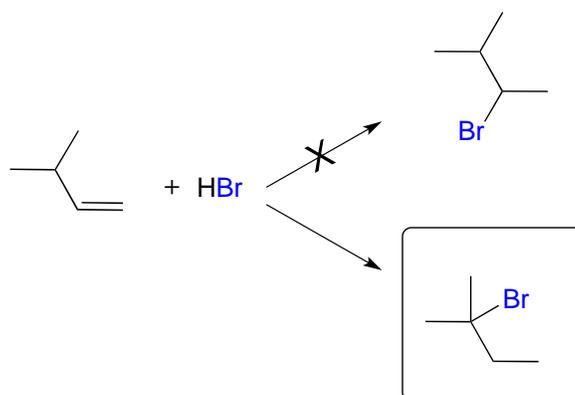


b) (*challenging!*) Ring-deactivating substituents are usually also *meta*-directing. Use one of your carbocation intermediate drawings from part (a) of this exercise, and the concept of resonance, to explain this observation.

c) (*answer part (b) first*) Look again at the vitamin K biosynthesis reaction, and discuss the ring activating/directing effects of the two substituents on the substrate.

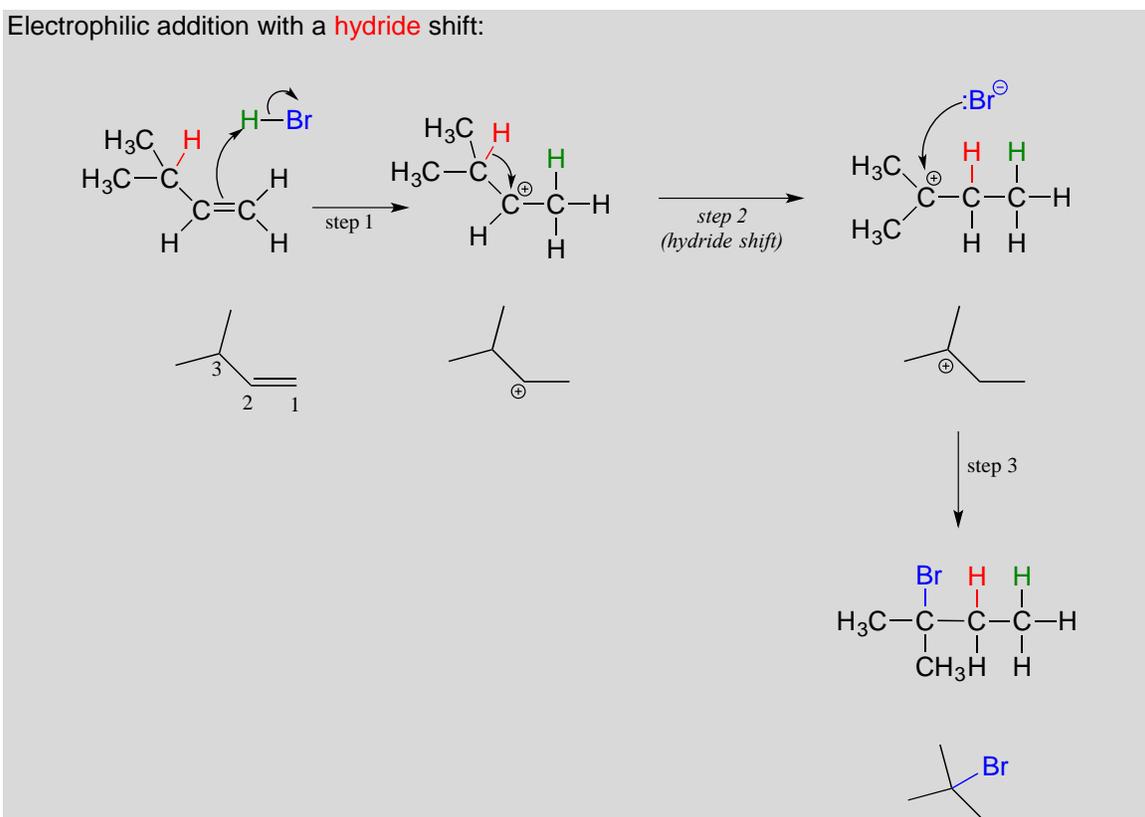
Section 14.5: Carbocation rearrangements

Earlier in this chapter we introduced the so-called 'Markovnikov rule', which can be used to predict the favored regiochemical outcome of electrophilic additions to asymmetric alkenes. According to what we have learned, addition of HBr to 3-methyl-1-butene should result in a secondary bromoalkane. However, the predominant product that is actually observed in this reaction is a *tertiary* alkyl bromide! Little or no secondary alkyl bromide forms.



To explain this result, let's take a look at the mechanism for the reaction:

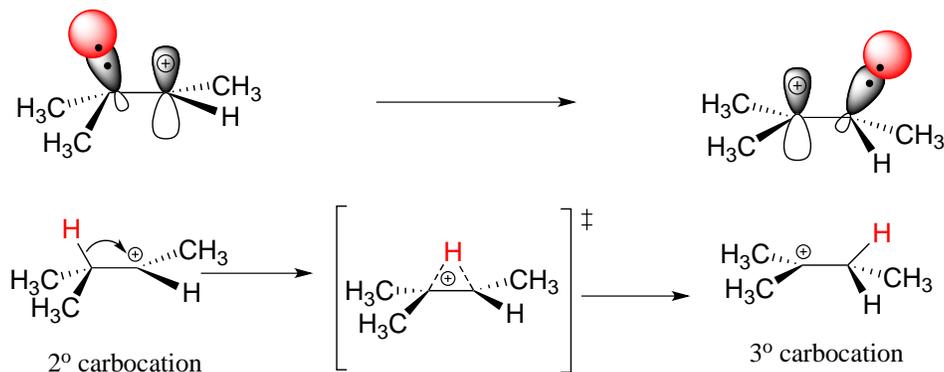
Electrophilic addition with a **hydride** shift:



Protonation of the double bond results in a secondary carbocation (step 1). What happens next (step 2 above) is a process called a **carbocation rearrangement**, and more specifically, a **hydride shift**. The electrons in the bond between carbon #3 and a hydrogen are attracted by the positive charge on carbon #2, and they simply shift over to fill the empty p orbital, pulling the proton over with them. Notice that the hydride, in shifting, is *not* acting as an actual leaving group - a hydride ion is a very strong base and a very poor leaving group.

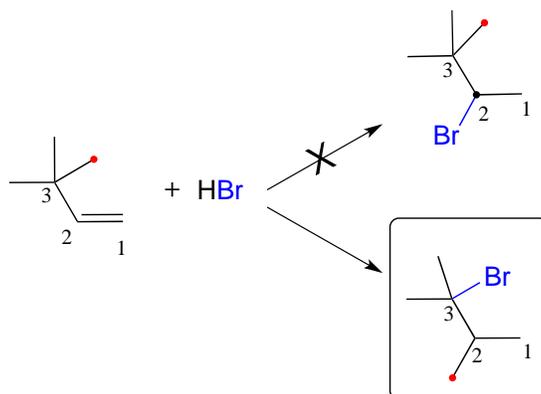
An important reminder: a hydride ion (H^-) is a proton *plus two electrons*. Be sure not to confuse a hydride ion with H^+ , which is *just* a proton without any electrons.

As the shift proceeds, a new C-H σ bond is formed at carbon #2, and carbon #3 is left with an empty p orbital and a positive charge.



What is the thermodynamic driving force for this process? Notice that the hydride shift results in the conversion of a secondary carbocation to a (more stable) tertiary carbocation - a thermodynamically *downhill* step. As it turns out, the shift occurs so quickly that it is accomplished before the bromide nucleophile has time to attack at carbon #2. Rather, the bromide will attack (step 3) at carbon #3 to complete the addition.

Consider another example. When HBr is added to 3,3-dimethyl-1-butene, the product is a tertiary - rather than a secondary - alkyl bromide.

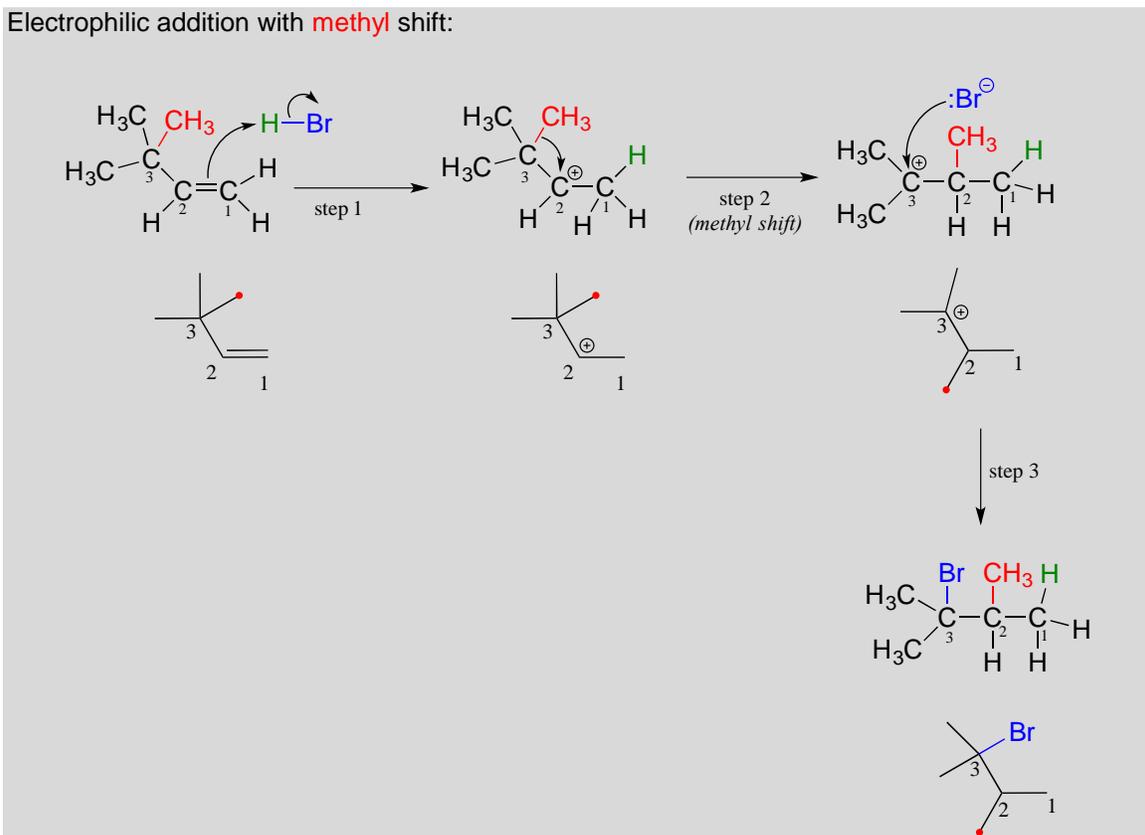


Notice that in the observed product, *the carbon framework has been rearranged*: the methyl carbon indicated by a red dot has shifted from carbon #3 to carbon #2. This is an example of another type of carbocation rearrangement, called a **methyl shift**.

Below is the mechanism for the reaction. Once again a secondary carbocation intermediate is formed in step 1. In this case, there is no hydrogen on carbon #3 available to shift over create a more stable tertiary carbocation. Instead, it is a methyl group that

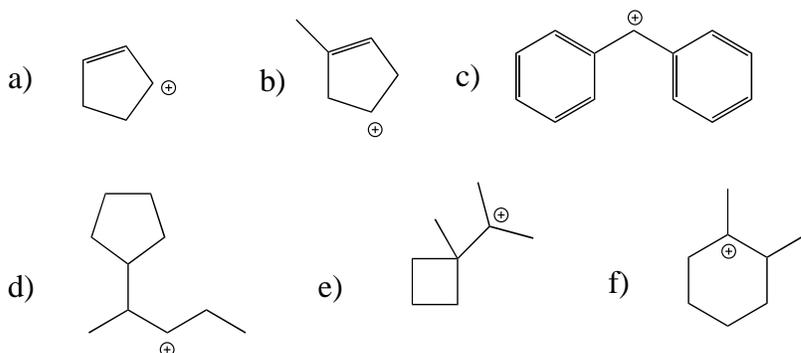
does the shifting, as the electrons in the carbon-carbon σ bond move over to fill the empty orbital on carbon #2 (step 2 below).

Electrophilic addition with **methyl** shift:



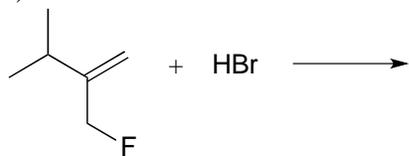
The methyl shift results in the conversion of a secondary carbocation to a more stable tertiary carbocation. The end result is a rearrangement of the carbon framework of the molecule.

Exercise 14.21: Which of the following carbocations are likely to undergo a shift? If a shift is likely, draw the new carbocation that would result.

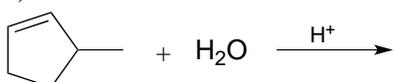


Exercise 14.22: In the (non-biochemical) reactions below, the major product forms as the result of a hydride or methyl shift from a carbocation intermediate. Predict the structure of the major product for each reaction, disregarding stereochemistry.

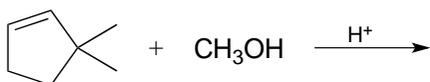
a)



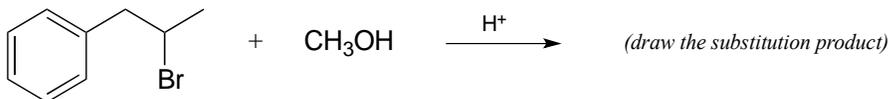
b)



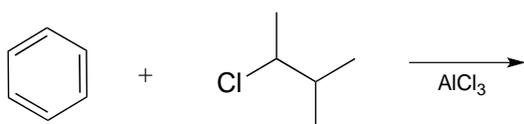
c)



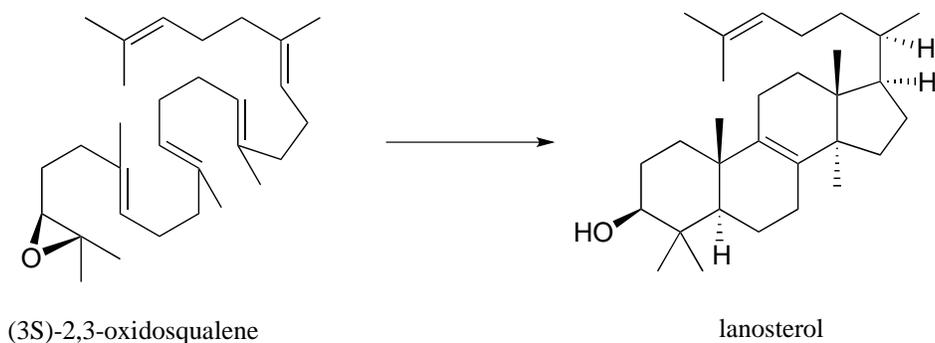
d)



Exercise 14.23: Draw the most abundant product of this laboratory Friedel-Crafts reaction:



Carbocation rearrangements are involved in many known biochemical reactions. Rearrangements are particularly important in carbocation-intermediate reactions in which isoprenoid molecules cyclize to form complex multi-ring structures. For example, one of the key steps in the biosynthesis of cholesterol is the electrophilic cyclization of oxidosqualene to form a steroid called lanosterol (E.C. 5.4.99.7).



This complex but fascinating reaction has two phases. The first phase is where the actual cyclization takes place, with the formation of four new carbon-carbon bonds and a carbocation intermediate. This phase is a 'cascade' of electrophilic alkene addition steps, beginning with addition of an electrophilic functional group called an 'epoxide'.

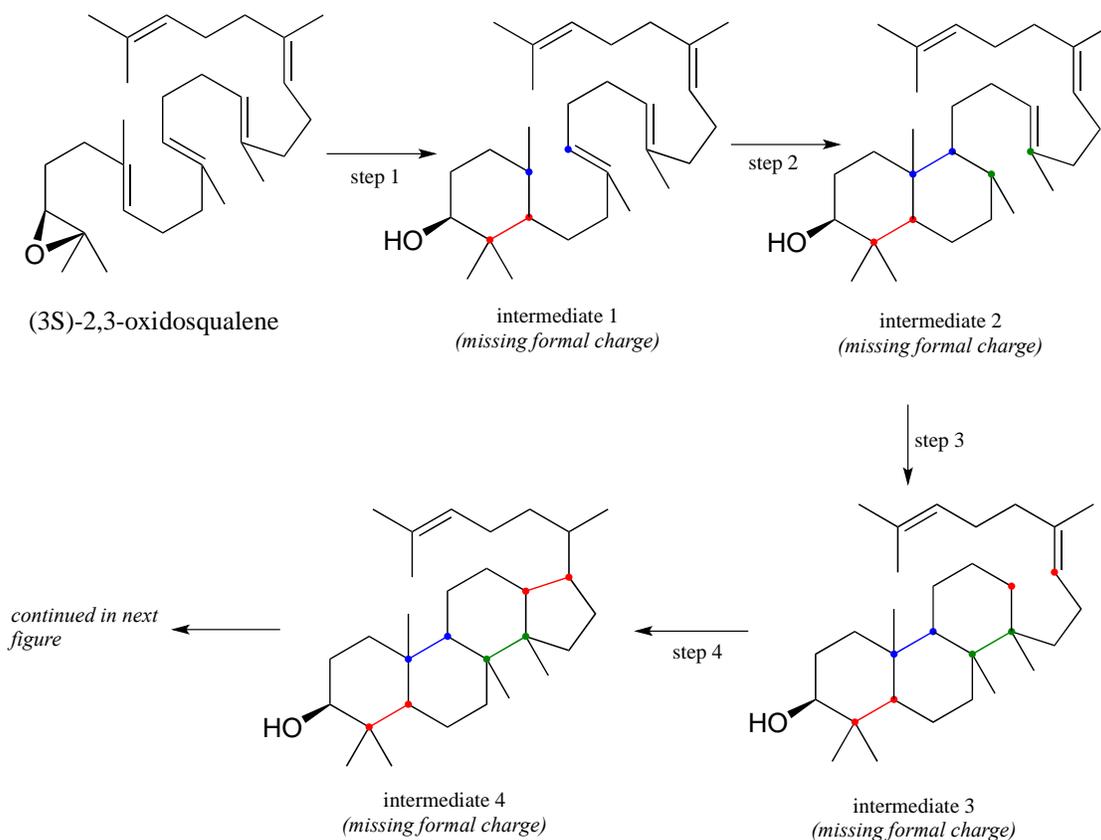
The epoxide functional group - composed of a three membered ring with two carbons and an oxygen - is relatively rare in biomolecules and biochemical reactions, and for this reason it is not discussed in detail in this book. However, epoxides are an important and versatile intermediate in laboratory organic synthesis, so you will learn much more about how they are made and how they react if you take a course in chemical synthesis. For now, it is sufficient to recognize that the carbon atoms of an epoxide are potent electrophiles, due to both the carbon-oxygen bond dipoles and the inherent strain of the three membered ring.

The second phase involves a series of hydride and methyl shifts culminating in a deprotonation. In the exercise below, you will have the opportunity to work through the entire cyclase reaction mechanism. In section 15.7, we will take a look at how the epoxide group of oxidosqualene is formed.

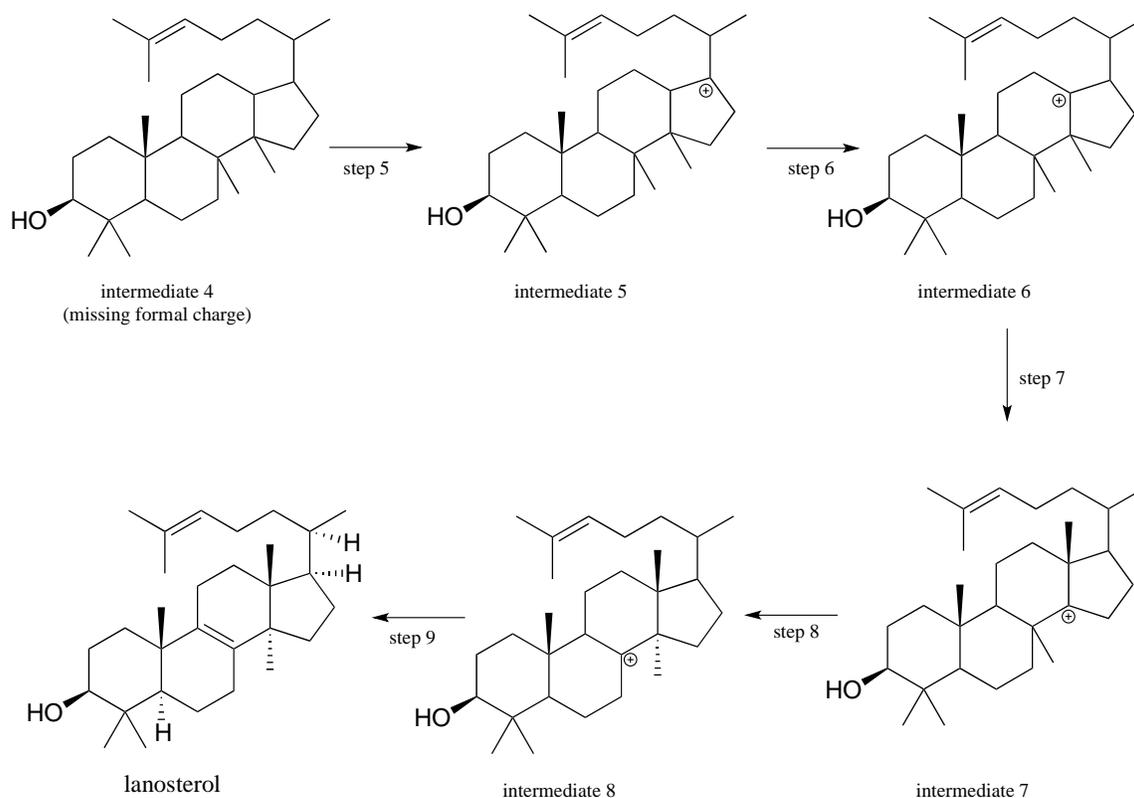
Exercise 14.24:

a) The figure below outlines the first, cyclizing phase of the reaction that converts oxidosqualene to lanosterol. However, the diagram is missing electron movement arrows, and intermediates 1-4 are all missing formal charges - fill these in.

First phase (ring formation):



b) Next comes the 'shifting' phase of the reaction. Once again, supply the missing mechanistic arrows.

Second phase: rearrangement and deprotonation

c) Look at the first and last steps of the entire process: overall, would you describe this as an electrophilic addition or substitution?

The oxidosqualene cyclization reaction and others like it are truly remarkable examples of the exquisite control exerted by enzymes over the course of a chemical reaction. Consider: an open-chain starting molecule is converted, by a single enzyme, into a complex multiple fused-ring structure with *seven* chiral centers. Oxidosqualene could potentially cyclize in many different ways, resulting in a great variety of different products. In order for the enzyme to catalyze the formation of a *single* product with the correct connectivity and stereochemistry, the enzyme must be able to maintain precise control of the conformation of the starting compound and all reactive intermediates in the active site, while also excluding water molecules which could attack at any of the positively charged carbons.

Key learning objectives for this chapter

Understand why the π bond in a carbon-carbon double bond is more reactive than the σ bond.

Addition

Be able to draw a mechanism for the electrophilic addition of a haloacid to an alkene.

Stereochemistry: understand why nonenzymatic electrophilic addition of a haloacid to an alkene occurs with racemization (both inversion and retention of configuration) at both alkene carbons. Be able to distinguish *syn* vs *anti* addition.

Regiochemistry: Be able to predict the regiochemical outcome of an electrophilic addition, based on the relative stability of the two possible carbocation intermediates. Be able to predict when anti-Markovnikov addition is likely to occur.

Be able to predict the product of nonenzymatic addition of water/alcohol to an alkene, including regio- and stereo-chemistry when applicable. Be able to draw complete mechanisms.

Be able to predict the products of nonenzymatic addition of water/alcohol to a conjugated diene or triene, including regio- and stereochemistry when applicable. Be able to draw complete mechanisms, including multiple resonance forms for carbocation intermediates.

Be able to apply your understanding of nonenzymatic alkene addition reactions to draw mechanisms for enzymatic addition reactions. In particular, you should be able to draw mechanisms for biochemical electrophilic addition reactions in which a new carbon-carbon bond is formed.

Elimination

Be able to draw a mechanism for an E1 elimination reaction.

Be able to predict possible E1 reaction products from a common starting compound, taking into account both regiochemistry (Zaitsev's rule) and stereochemistry.

Be able to recognize and draw a mechanism for biochemical E1 reactions in which

- a) the second step is a deprotonation event
- b) the second step is a decarboxylation event

Be able to distinguish whether a biochemical elimination reaction is likely to proceed through a E1cb or E1 mechanism, based on the structure of the starting compound.

Isomerization/substitution

Be able to recognize and draw mechanisms for a biochemical electrophilic isomerization reaction (shifting the location of the carbon-carbon double bond).

Be able to recognize and draw mechanisms for a biochemical electrophilic substitution reaction.

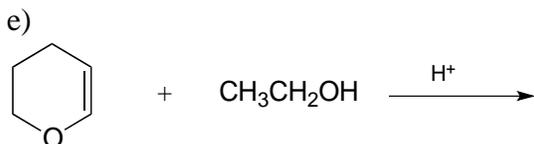
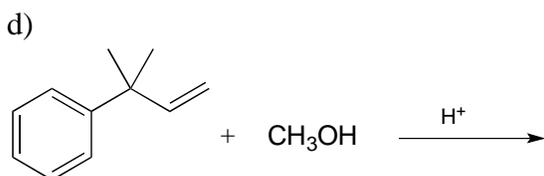
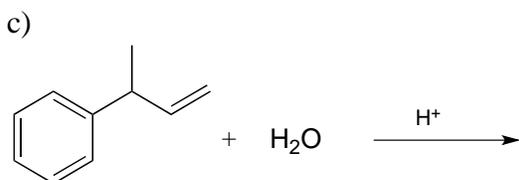
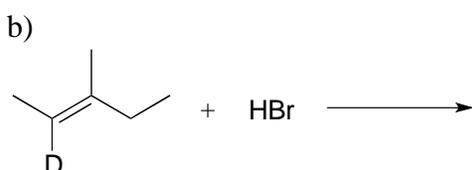
Be able to recognize and draw mechanisms for a biochemical electrophilic *aromatic* substitution reaction, and be able to explain the ring-activating effect (how the carbocation intermediate is stabilized by resonance, usually with lone-pair electrons on either an oxygen or a nitrogen atom).

Be able to recognize when a hydride or alkyl shift is likely to occur with a carbocation reaction intermediate.

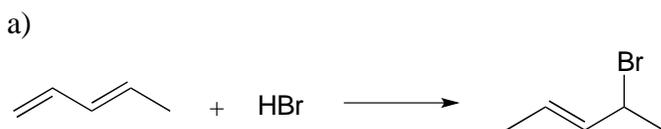
Be able to draw a mechanism for a reaction that includes a carbocation rearrangement.

Problems

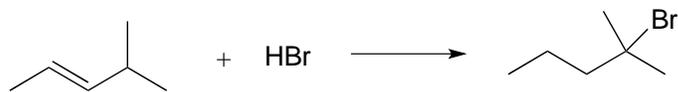
P14.1: Draw the major product(s) (including all stereoisomers) that would be expected to result from the nonenzymatic electrophilic addition reactions below. Your product(s) should result from the most stable possible carbocation intermediate. Hint: consider the possibility of thermodynamically favorable rearrangement steps.



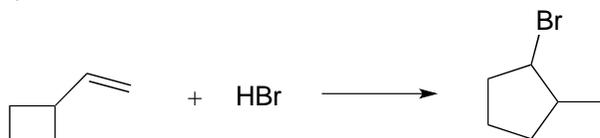
P14.2: Draw likely mechanisms for the nonenzymatic reactions below. Products shown are not necessarily the most abundant for the reaction.



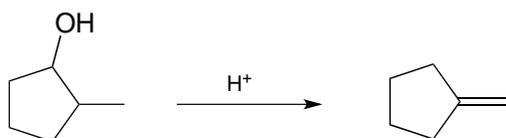
b)



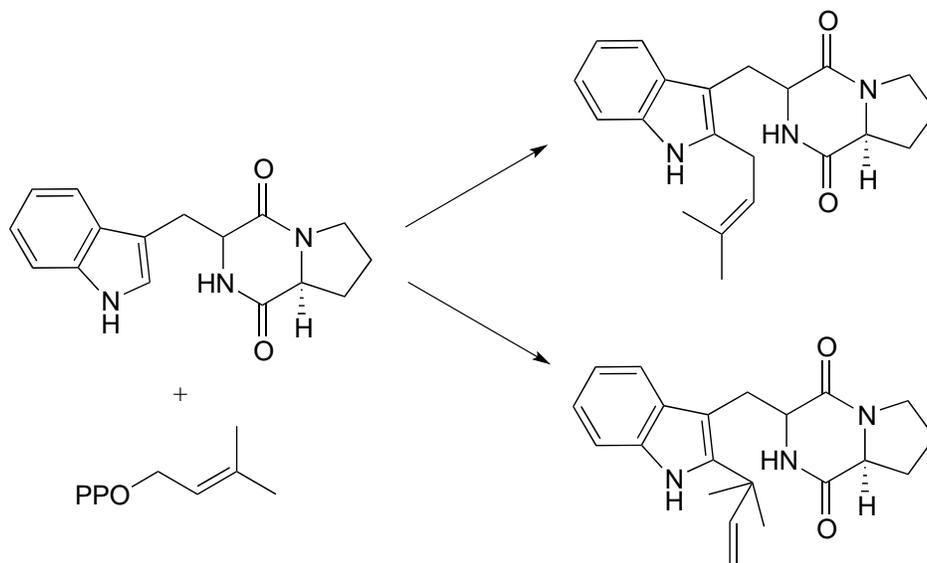
c)



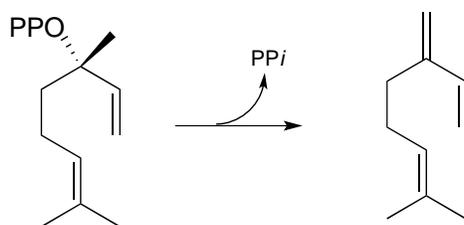
d)



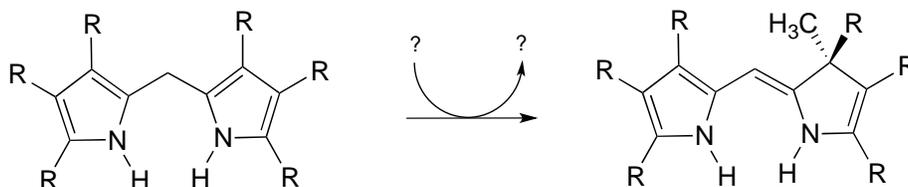
P14.3: Provide mechanisms for the following reactions, both of which are part of an alkaloid synthesis pathway in fungi. (*Microbiol.* **2005**, 151, 2199)



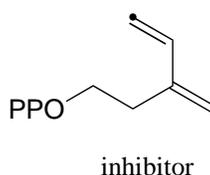
P14.4: Draw a likely mechanism for the reaction below. The product is myrcene, a compound produced by fir trees as a defense against insects. (*J. Biol. Chem* **1997**, 272, 21784)



P14.5: Provide a mechanism for the following reaction from the vitamin B₁₂ biosynthetic pathway, and identify the missing participants indicated by question marks in the figure.

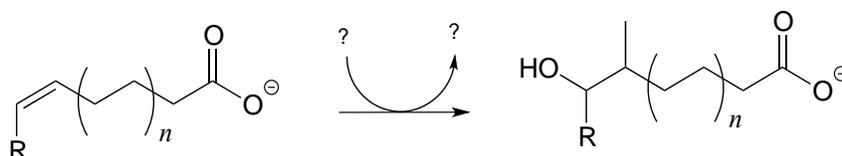


P14.6: A diene molecule synthesized in the laboratory was found to irreversibly inhibit the action of isopentenyl diphosphate isomerase (section 14.3) when the carbon indicated with a dot becomes covalently bonded to a cysteine residue in the enzyme's active site. Propose a mechanism showing how this could happen. (*J. Am. Chem. Soc.* **2005**, 127, 17433)

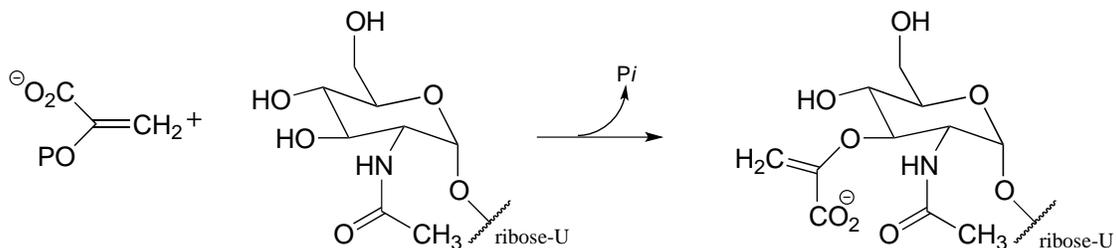


P14.7: Nonenzymatic electrophilic addition of water to alkynes results in the formation of a ketone or an aldehyde, depending on the starting alkyne. A vinylic carbocation is a key intermediate, and the reaction is accelerated with the use of a catalytic amount of strong acid. Predict the product the addition of water to propyne, and draw a mechanism for the reaction.

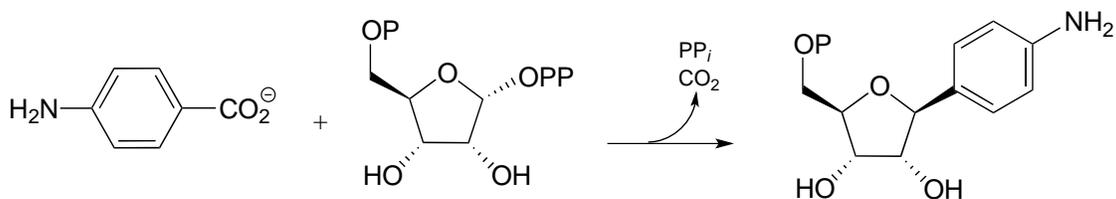
P14.8: The reaction below is part the pathway by which some bacteria -including the species which cause tuberculosis and leprosy - form distinctive branched-chain fatty acids for incorporation into their cell walls. This enzyme is of interest to scientists as possible targets for new antibiotic drugs. Propose a likely mechanism, and identify the missing participants denoted by question marks. (*J. Biol. Chem.* **2006**, 281, 4434)



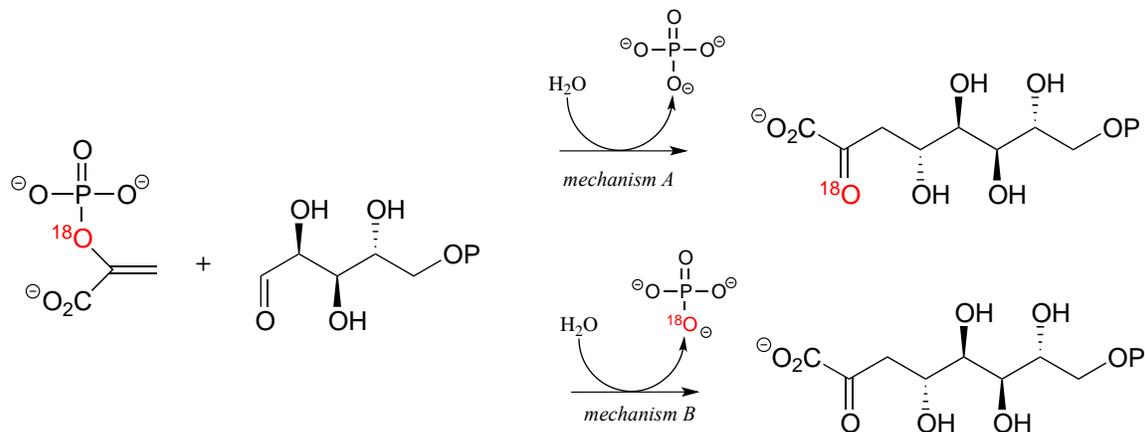
P14.9: Suggest a likely mechanism for this reaction, which is a key step in the synthesis of bacterial cell walls. Your mechanism should show an electrophilic addition, followed by an E1 elimination.



P14.10: Suggest a mechanism for the following reaction, which is part of the pathway by which many microbes synthesize methanopterin, a derivative of the vitamin folic acid. *Hint:* the mechanism can be described as an electrophilic aromatic substitution with a final decarboxylation step in place of the usual deprotonation step. (*J. Biol. Chem.* **2004**, 279, 39389).



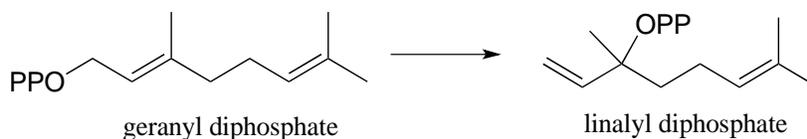
P14.11: Researchers investigated the mechanism of the enzyme 3-deoxy-D-manno-octulosonate-8-phosphate synthase by running the reaction with one of the substrates labeled with the ^{18}O isotope (colored red in the scheme below). Consider the two hypothetical results shown below, each pointing to a different mechanism. Both mechanisms involve a carbocation intermediate. (*Biochem. Biophys. Res. Commun.* **1988**, 157, 816)



a) Propose a mechanism that is consistent with result A, in which the ^{18}O label ends up in the ketone group of the organic product.

b) Propose a mechanism that is consistent with result B, in which the ^{18}O label ends up in the inorganic phosphate by-product.

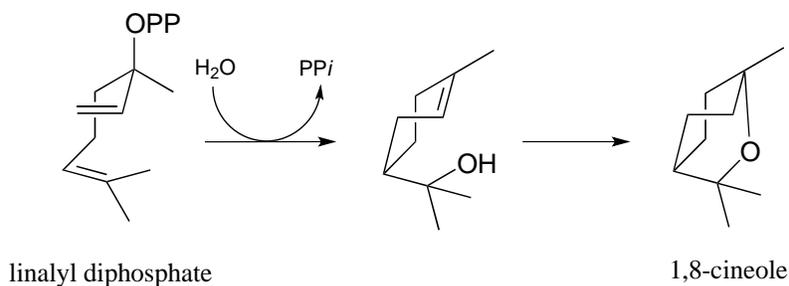
P14.12: Consider the following isomerization reaction (*J. Biol. Chem.* **1989**, 264, 2075):



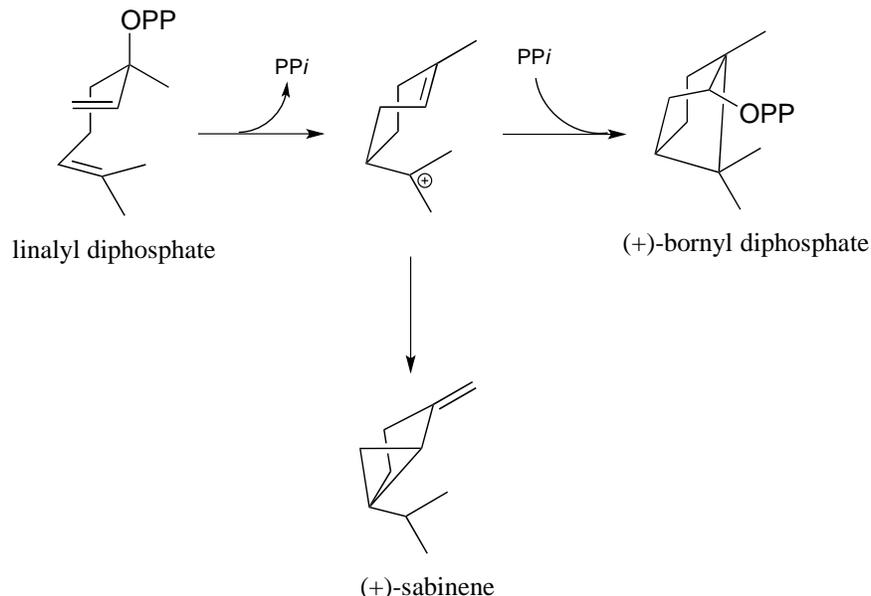
a) Suggest a likely mechanism involving a carbocation intermediate.

b) Suggest an isotopic labeling experiment (using substrate labeled with ^{18}O) that could confirm or rule out an alternative, *concerted* isomerization mechanism (*ie.* one without formation of a carbocation intermediate). Explain your reasoning.

c) Propose a mechanism for the following reaction (notice that the starting compound is linalyl diphosphate from part (a), drawn in a different conformation). (*Arch Biochem Biophys* **2003**, 417, 203)



For parts d-f, refer to the figure below:

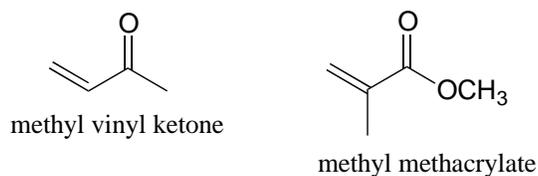


d) Provide mechanisms for the conversion of linalyl diphosphate to (+)-bornyl diphosphate

e) Provide mechanisms for the conversion of linalyl diphosphate to (+)-sabinene.

f) Is the second step in the (+)-bornyl diphosphate pathway (addition of phosphate) a Markovnikov or anti-Markovnikov addition? Explain the regiochemistry of this step in terms of carbocation stability.

P14.13: The two compounds shown below were each treated with HBr, and the products isolated and analyzed by ^1H NMR. Use the NMR data provided to determine the structure of both products, then explain the observed regiochemistry of the addition reaction.



^1H -NMR data for product of HBr addition to methyl vinyl ketone:

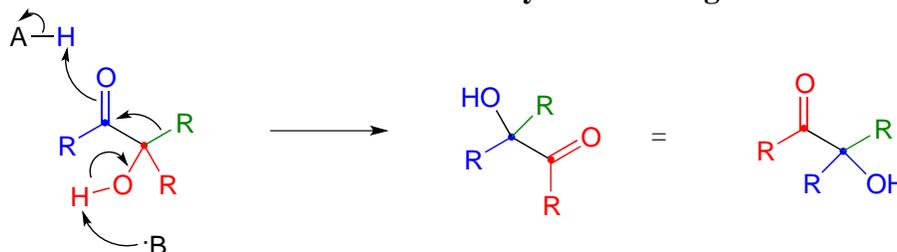
δ	integration	splitting
2.2	1.5	s
3.0	1	t
3.5	1	t

$^1\text{H-NMR}$ data for product of HBr addition to methyl methacrylate:

δ □ □ □ □ □ □ integration splitting

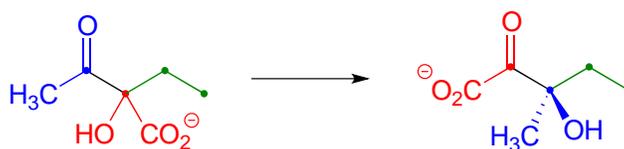
1.3	3	d
2.3	1	sextet
3.5	2	d
3.7	3	s

P14.14: Ketones and aldehydes with a hydroxy group in the α position are known to undergo an isomerization reaction known as an **acyloin rearrangement**:

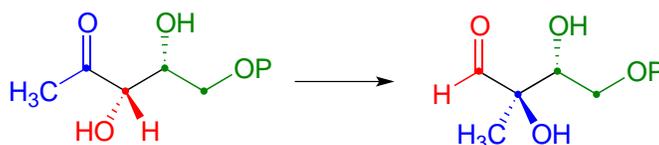


Notice in the general acyloin rearrangement mechanism below, the green alkyl group is *shifting* from the α carbon (red) to the carbonyl carbon (blue). Notice also that this shift does not involve a carbocation intermediate, although a resonance contributor can be drawn in which the carbonyl carbon has a positive charge.

a) Draw a mechanism for this acyloin rearrangement step in the biosynthetic pathway for the amino acid leucine:

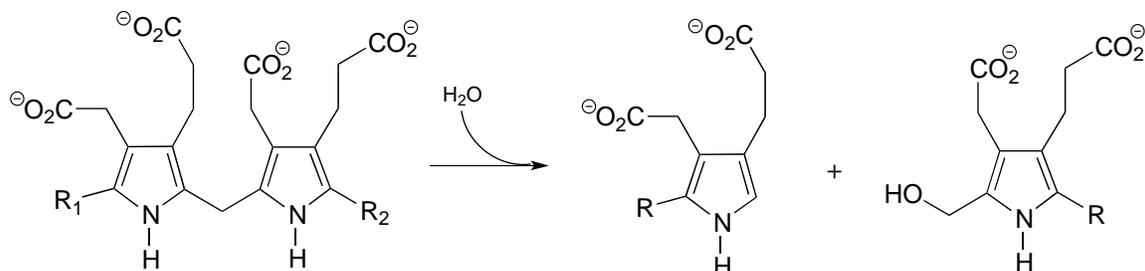


b) Draw a mechanism for this acyloin rearrangement step in the isoprenoid biosynthetic pathway in bacteria:

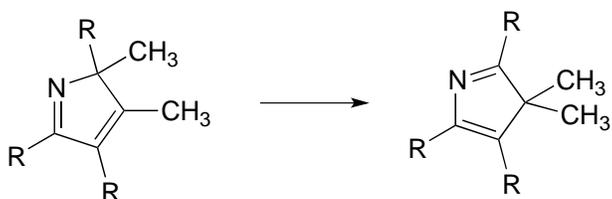


P14.15: Propose mechanisms for these reactions in the vitamin B₁₂ biosynthetic pathway:

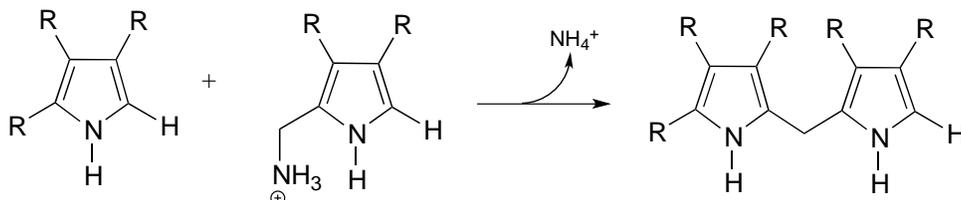
a)



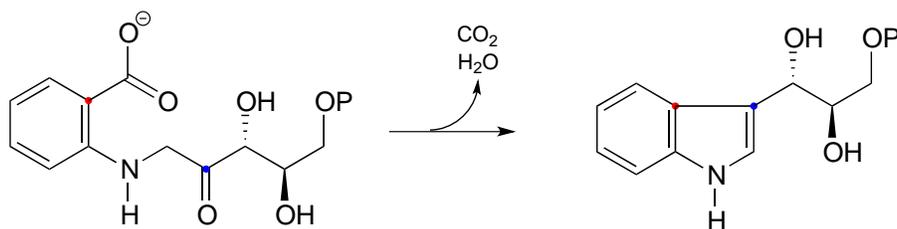
b)



c)



P14.16: An early reaction in the biosynthesis of tryptophan can be described as an intramolecular electrophilic aromatic substitution/decarboxylation hybrid, followed by an E1 dehydration (EC 4.1.1.48).

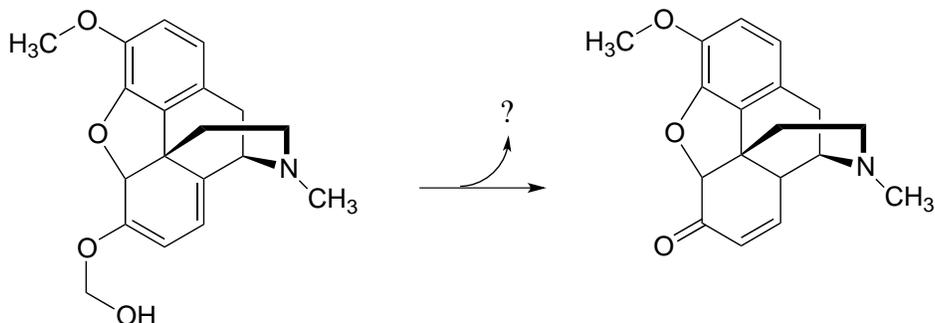


a) Draw a mechanism that corresponds to the verbal description given above. Use resonance structures to show how the nitrogen atom helps to stabilize the carbocation intermediate. *Hint:* the electrophilic carbon in this case is a ketone rather than a carbocation.

b) What aspect of this reaction do you think helps to compensate for the energetic disadvantage of not having a powerful carbocation electrophile?

c) Again thinking in terms of energetics, what is the 'driving force' for the dehydration step?

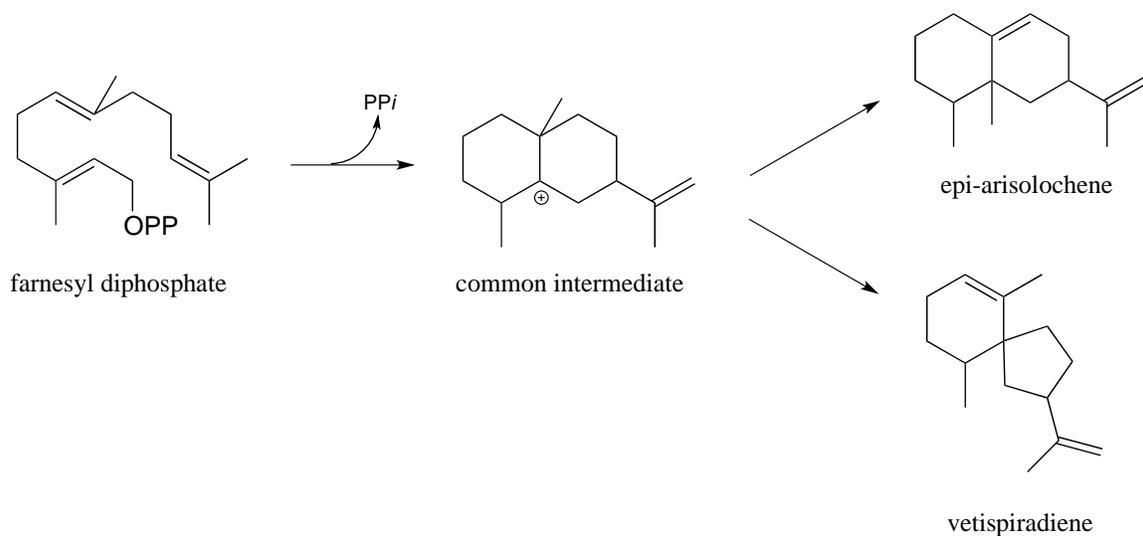
P14.17: Propose a likely carbocation-intermediate mechanism for the following reaction in the biosynthesis of morphine, being sure to identify the structure of the organic compound released in the reaction.



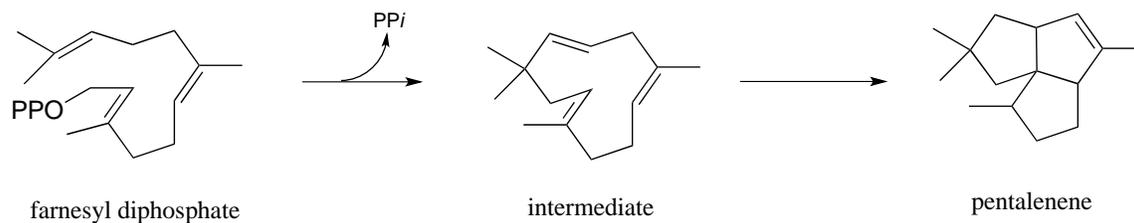
P14.18: Propose mechanisms for these three electrophilic cyclization reactions. Carbocation rearrangement steps are involved.

a) epi-aristolochene

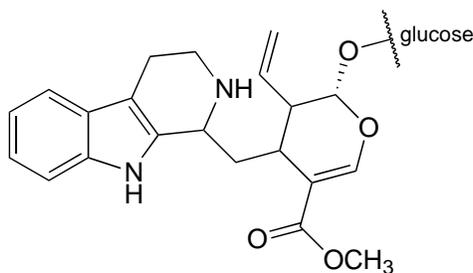
b) vetispiradiene (*Science* **1997**, 277, 1815)



c) pentalenene (*Science* **1997**, 277, 1820)



P14.19: Strictosinide, an intermediate in the biosynthesis of the deadly poison strychnine, is formed from two steps: a) intermolecular imine formation, and b) an intramolecular, ring-forming electrophilic aromatic substitution with the imine carbon from step (a) as the electrophile.

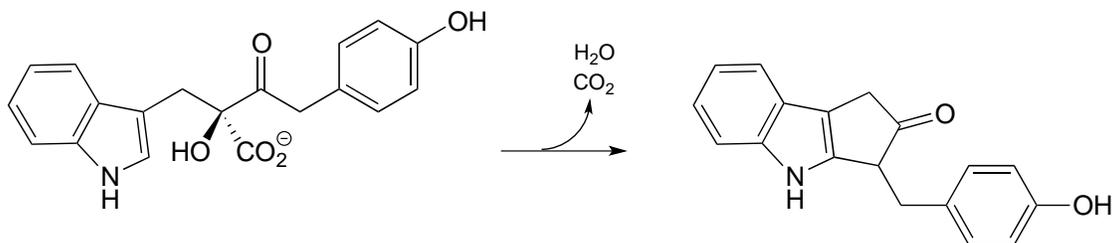


strictosinide

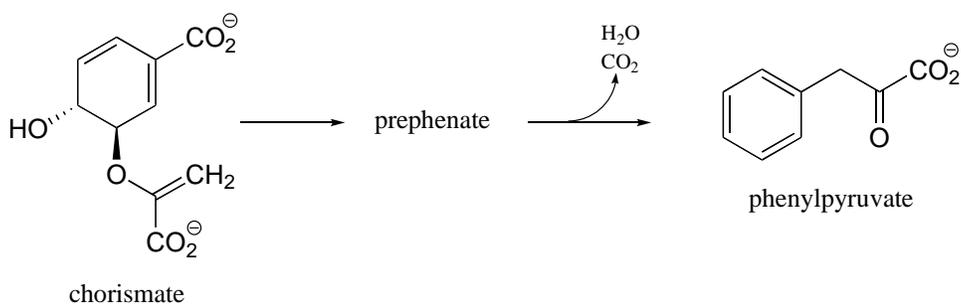
Given this information, predict the two precursors to strictosinide, and draw a mechanism for the reaction described. Hint: use the 'retro' skills you developed in chapters 12 and 13.

P14.20: In the introduction to chapter 8, we learned about reactions in which the cytosine and adenine bases in DNA are methylated. In the course of that chapter, we learned how adenine N-methylation occurs in bacteria, but we were not yet equipped to understand cytosine C-methylation, which was the more relevant reaction in terms of human health and development. Now we are: propose a reasonable mechanism for the C-methylation of cytosine.

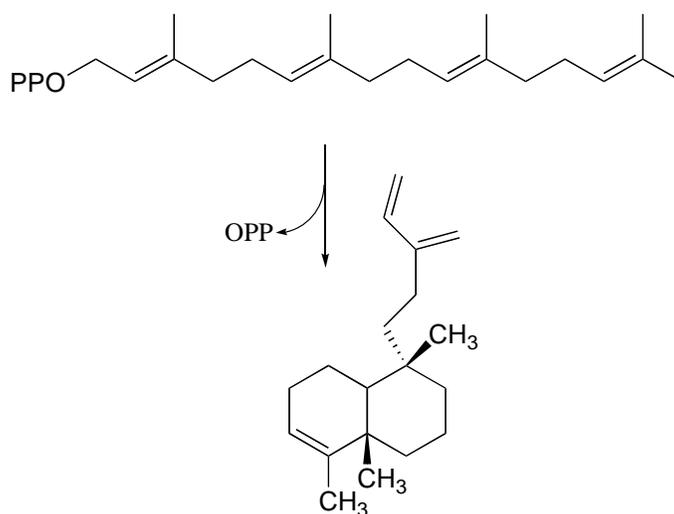
P14.21: The reaction below has been proposed to proceed via a cyclization step followed by an E1/decarboxylation step (in other words, an E1 mechanism where decarboxylation occurs instead of deprotonation). Draw a mechanism that fits this description, and show the most stable resonance contributors of the two key cationic intermediates.



P14.22: The conversion of chorismate to phenylpyruvate is a key transformation in the biosynthesis of phenylalanine. The first step is a concerted electrophilic rearrangement to form prephenate (this step involves a six-membered transition state). Deduce the structure of prephenate, and provide a complete mechanism for the transformation.



P14.23: Suggest likely a mechanism for the following reaction:



Chapter 15

Oxidation and reduction reactions



photo credit: Johann Schwarz (<https://www.flickr.com/photos/blacksportsphotos/>)

Introduction

Theo Ross was not doing very well at his summer job, and he was frustrated. His boss had given him specific instructions, and yet Theo kept botching the job, over and over again. Theo was not used to failure – he had achieved almost perfect scores on both the ACT and SAT college entrance exams, and was headed to Stanford University in the fall. Why couldn't he get it right? It wasn't brain surgery, after all.

Well, actually – it *was* brain surgery.

An [April 17, 2014 article in *Sports Illustrated*](#) tells Theo's story. Wanting to do something interesting over the summer of 2010 before he started college, Theo had applied for a research internship at the National Institutes of Health in Bethesda, Maryland. This was an extremely competitive program normally reserved for outstanding college students, but somehow Theo had managed to win a coveted spot in the program, working with Dr. Dorian McGavern, a neurologist studying how meningitis effects the brain. Dr. McGavern assigned Theo the task of performing 'skull-thinning' surgery on mice, part of which involved using a special saw to shave down a small section of the bone in order to gain access to the brain. It was a delicate procedure, something that even some experienced neurosurgeons who had tried it had found challenging. Any small slip resulted in a concussion to the mouse's brain, rendering it useless for the study. Theo just couldn't get the hang of it, and ended up concussing one mouse after another.

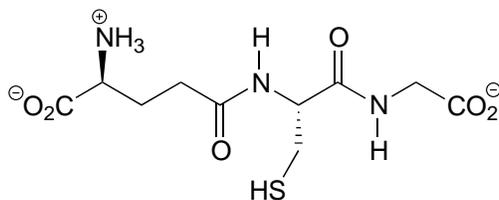
You have probably heard the old expression: “when life gives you lemons, make lemonade”. Theo made a lot of lemonade that summer.

Theo and Dr. McGavern eventually realized that his failure at the procedure actually presented an opportunity to observe what happens to a brain right after a concussive injury. Theo started doing more skull-thinning surgeries, but now the goal was to *cause* concussion, rather than to avoid it. The concussed mice (who had been anaesthetized prior to the surgery) were immediately strapped under a microscope so that Theo could observe how their brains responded to the injury. This was new, and very exciting stuff: most of what neurologists knew about concussions up to that point had come from MRI (magnetic resonance imaging – see chapter 5) or autopsies. Nobody knew very much about what happens at the cellular level in a brain in the minutes and hours after a concussion has occurred. In addition, the problem of traumatic head injuries and the long-lasting effects they cause was becoming an increasingly hot topic in the news, critically relevant to thousands of veterans returning from Iraq and Afghanistan as well as to football players and other athletes in contact sports– including Theo, who had been a competitive wrestler in high school. (You might have been wondering why Theo's story appeared in *Sports Illustrated* – now you know.)

Theo spent the rest of that summer, and every spring break and summer vacation over the next few years, working in McGavern's lab on the new project. He and McGavern found evidence that the 'hidden' damage to a concussed brain – that which went undetected in MRI scans but could come back to haunt the victim years later in the form of recurring headaches, memory loss, and depression – may be caused by a type of molecule referred to as 'reactive oxygen species', or ROS, leaking from damaged tissues into the brain. ROS are potentially harmful byproducts of respiration such as hydrogen peroxide (H_2O_2) that are constantly being produced in our cells. Although ROS can cause serious oxidative damage if they are allowed to build up, our bodies have evolved ways to deal with them, using so-called 'ROS scavengers' to convert them to something innocuous like water.

With this new understanding, Theo and his mentor had another idea: what if they could prevent the ROS from causing further damage to a recently concussed brain by applying an scavenger to the injury? After some trial and error, they found that an ROS scavenger

compound called glutathione, when applied directly to the skull of a concussed mouse within a few minutes to three hours after the injury, could permeate the bone and react with the ROS. Brain cells from these glutathione-treated mice appeared normal, with none of the signs of ROS damage Theo was used to seeing.



glutathione

The road from an initial scientific discovery to a safe and effective medical treatment is often a very long one, but Theo Roth and Dorian McGavern appear to have made a discovery that could eventually help prevent some of the most devastating and long-term damage caused by traumatic head injuries. In the end, it's a very good thing that Theo's hands were not cut out for brain surgery.

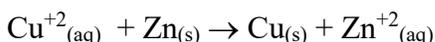
The chemistry of oxidation and reduction - often called 'redox' chemistry - is central to Theo Roth's discovery about what happens to a concussed brain at the molecular level. This chapter is dedicated to redox chemistry. We'll begin with a reminder of what you learned in General Chemistry about the fundamentals of redox reactions in the context of inorganic elements such as iron, copper and zinc: reduction is a gain of electrons, and oxidation is a loss of electrons. Then, we'll expand our understanding to include bioorganic redox reactivity, examining among other things how alcohols are converted to ketones and aldehydes, aldehydes are converted to carboxylic acids, and amines are converted to imines. We will also talk about redox reactions in the broader context of metabolism in living things.

A central player in some of the biochemical redox reactions we will see is the coenzyme called glutathione, Theo Roth's 'magic bullet' molecule that was able to rescue mouse brain cells from death by oxidation. We'll see how glutathione acts as a mediator in the formation and cleavage of disulfide bonds in proteins, and how it acts as an 'ROS scavenger' to turn hydrogen peroxide into water.

Section 15.1: Oxidation and reduction of organic compounds - an overview

You are undoubtedly already familiar with the general idea of oxidation and reduction: you learned in general chemistry that when a compound or element is oxidized it loses electrons, and when it is reduced it gains electrons. You also know that oxidation and reduction reactions occur in tandem: if one species is oxidized, another must be reduced at the same time - thus the term 'redox reaction'.

Most of the redox reactions you have seen previously in general chemistry probably involved the flow of electrons from one metal to another, such as the reaction between copper ion in solution and metallic zinc:



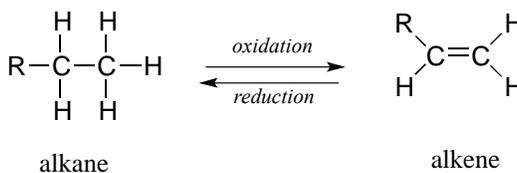
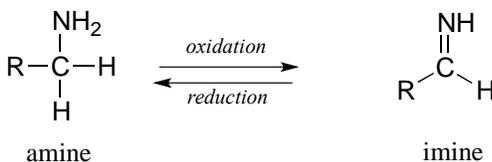
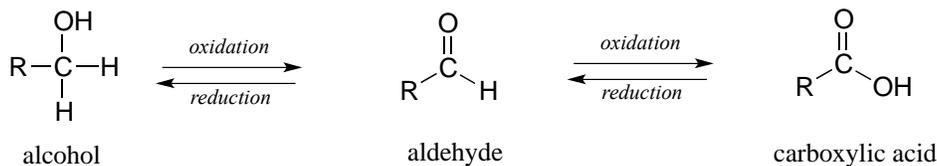
Exercise 15.1: Reading the reaction above from left to right, which chemical species is being oxidized? Which is being reduced?

When we talk about the oxidation and reduction of organic compounds, what we are mainly concerned with is the number of carbon-heteroatom bonds in the compound compared to the number of carbon-hydrogen bonds. (Remember that the term 'heteroatom' in organic chemistry generally refers to oxygen, nitrogen, sulfur, or a halogen).

Oxidation of an organic compound results an *increase* in the number of carbon-heteroatom bonds, and/or a *decrease* in the number of carbon-hydrogen bonds.

Reduction of an organic compound results in a *decrease* in the number of carbon-heteroatom bonds, and/or an *increase* in the number of carbon-hydrogen bonds.

Below are a number of common functional group transformations that are classified as redox.

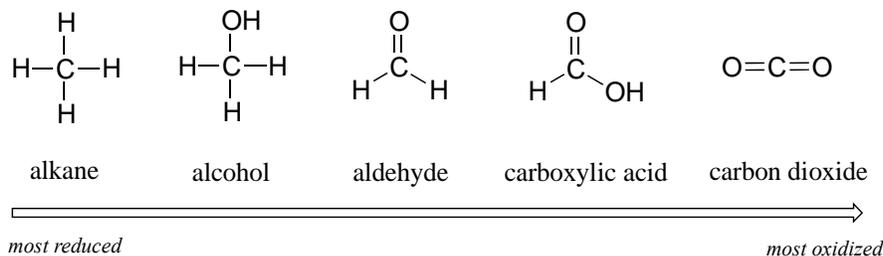


Heteroatoms such as oxygen and nitrogen are more electronegative than carbon, so when a carbon atom gains a bond to a heteroatom, it *loses* electron density and is thus being oxidized. Conversely, hydrogen is less electronegative than carbon, so when a carbon gains a bond to a hydrogen, it is *gaining* electron density, and thus being reduced.

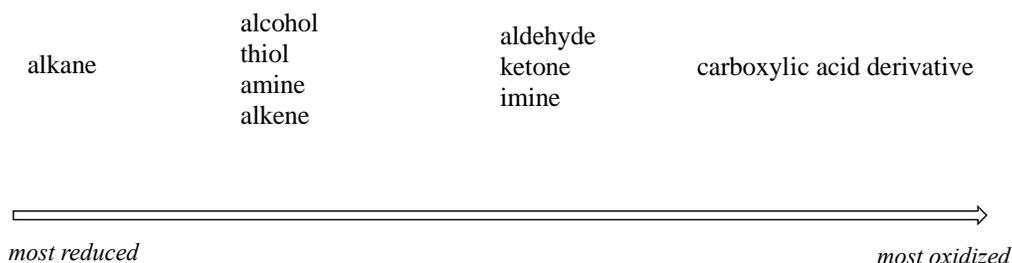
Exercise 15.2: The hydration of an alkene to an alcohol is *not* classified as a redox reaction. Explain.

For the most part, when talking about redox reactions in organic chemistry we are dealing with a small set of very recognizable functional group transformations. The concept of **oxidation state** can be useful in this context. When a compound has lots of carbon-hydrogen bonds, it is said to be in a lower oxidation state, or a more reduced state. Conversely, if it contains a lot of carbon-heteroatom bonds, it is said to be in a higher oxidation state.

We'll start with a series of single carbon compounds as an example. Methane, in which the carbon has four bonds to hydrogen, is the most reduced member of the group. The compounds become increasingly oxidized as we move from left to right, with each step gaining a bond to oxygen and losing a bond to hydrogen. Carbon dioxide, in which all four bonds on the carbon are to oxygen, is in the highest oxidation state.

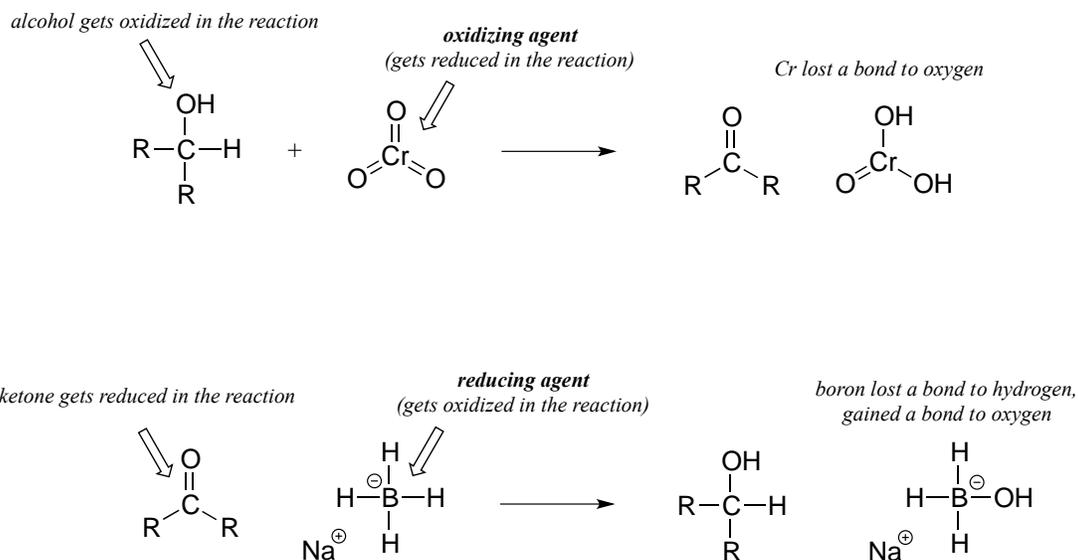


More generally, we can rank the oxidation state of common functional groups:



The alkane oxidation state is the most reduced. Alcohols, thiols, amines, and alkenes are all at the same oxidation state: therefore, a reaction converting one of these groups to another - an alcohol to alkene conversion, for example - is *not* a redox reaction. Aldehydes, however, are at a higher oxidation state than alcohols, so an alcohol to aldehyde conversion is an oxidation. Likewise, an imine to amine conversion is a reduction, but an imine to ketone conversion is not a redox reaction.

It is important to keep in mind that oxidation and reduction always occurs in tandem: when one compound is oxidized, another compound must be reduced. Often, organic chemists will use the terms **oxidizing agent** and **reducing agent** to refer to species that are commonly used, by human chemists or by nature, to achieve the oxidation or reduction of a variety of compounds. For example, chromium trioxide (CrO_3) is a laboratory oxidizing agent used by organic chemists to oxidize a secondary alcohol to a ketone, in the process being reduced to H_2CrO_3 . Sodium borohydride (NaBH_4) is a laboratory reducing agent used to reduce ketones (or aldehydes) to alcohols, in the process being oxidized to NaBH_3OH .

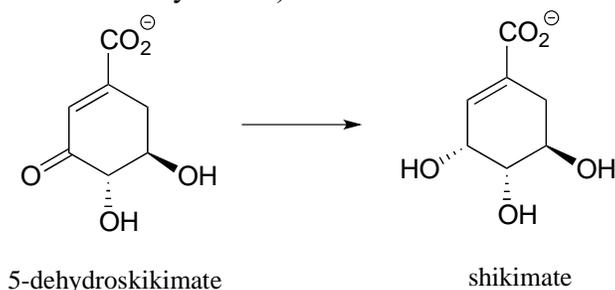


There is a wide selection of oxidizing and reducing agents available for use in the organic chemistry laboratory, each with its own particular properties and uses. For example, while sodium borohydride is very useful for reducing aldehyde and ketone groups to alcohols, it will not reduce esters and other carboxylic acid derivatives. If you take a course in synthetic organic chemistry, you will learn about the use of many of these agents.

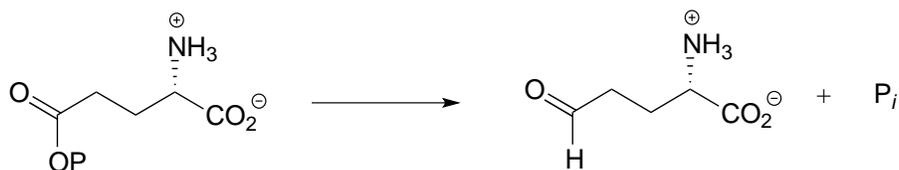
In this book, of course, we are concerned primarily with the organic chemistry that occurs within a living cell. A large part of this chapter will be spent looking at the action of two very important classes of coenzymes - the nicotinamides and the flavins - that serve as biochemical oxidizing and reducing agents. We also consider the oxidation and reduction of sulfur atoms in thiol groups, especially the thiol group on the side chain of cysteine residues in proteins.

Exercise 15.3: Each of the biochemical transformations shown below is a step in amino acid metabolism. For each, state whether the substrate is being oxidized, reduced, or neither oxidized nor reduced.

a) (from aromatic amino acid biosynthesis)



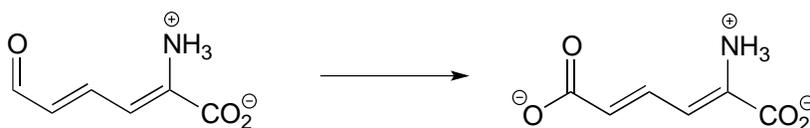
b) (from the biosynthesis of arginine and proline)



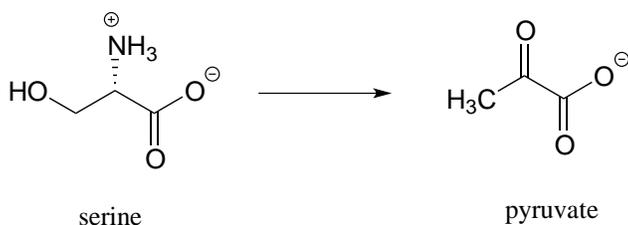
c) (from the catabolism of lysine)



d) (from the catabolism of tryptophan)

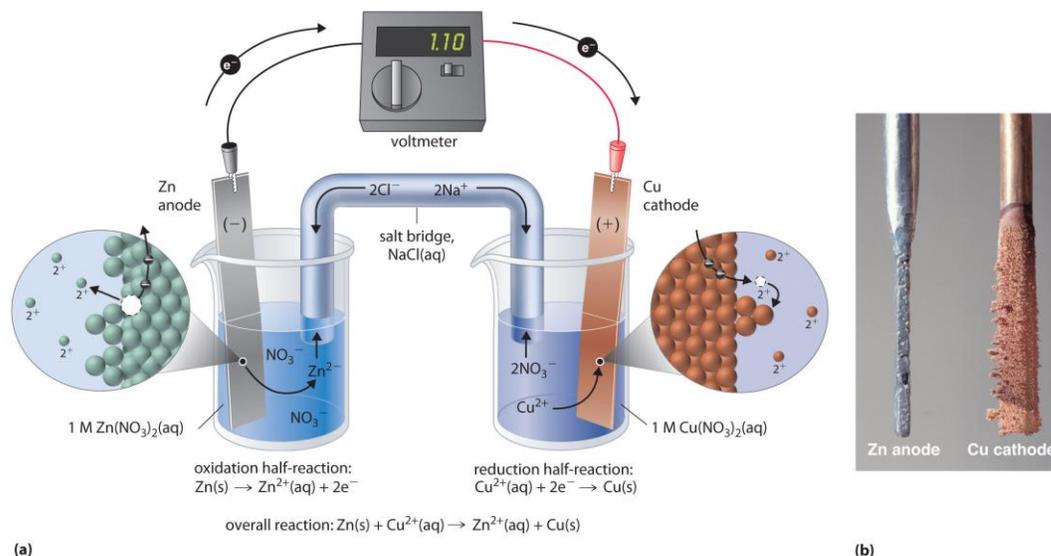


e) (from the catabolism of serine)



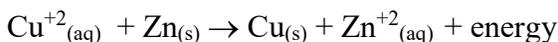
Section 15.2: Oxidation and reduction in the context of metabolism

Think back again to the redox chemistry that you learned in your general chemistry course. A common experiment in a general chemistry lab is to set up a galvanic cell consisting of a copper electrode immersed in an aqueous copper nitrate solution, connected by a wire to a zinc electrode immersed in an aqueous zinc nitrate solution.

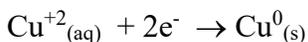


(<http://chemwiki.ucdavis.edu>)

When the cell is completed with a salt bridge, an electrical current begins to flow - what we have is a simple battery (figure a above). Over time, the copper electrode gets heavier as metallic copper is deposited on the copper cathode, while the zinc anode slowly dissolves into solution (figure b above). The redox reaction occurring here is:



Electrons flow from zinc metal to copper cations, creating zinc cations and copper metal: in other words, zinc metal is being oxidized to zinc cation and copper cation is being reduced to copper metal, as expressed by the two relevant half-cell reactions:

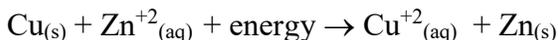
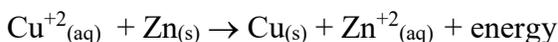


We can predict before we set up the cell that the spontaneous flow of electrons will go in the zinc to copper direction, just by looking at a table of **standard reduction potentials** (such a table was no doubt in your general chemistry text).

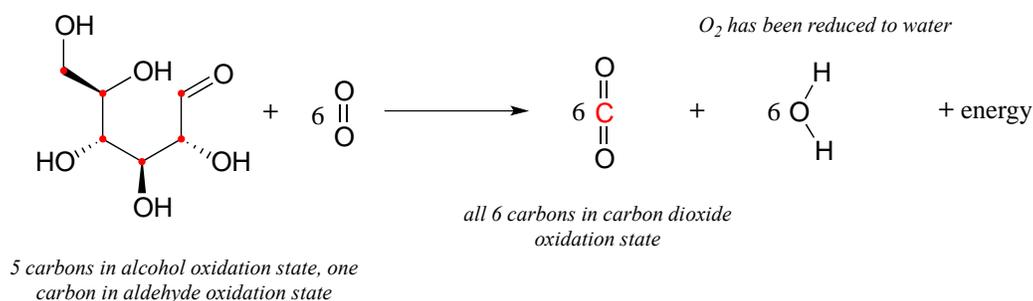
Standard reduction potentials at 25 °C

Reduction half-reaction	Reduction potential (volts)
$\text{Ag}^{+1}_{(\text{aq})} + \text{e}^{-} \rightarrow \text{Ag}^0_{(\text{s})}$	0.800
$\text{Cu}^{+2}_{(\text{aq})} + 2\text{e}^{-} \rightarrow \text{Cu}^0_{(\text{s})}$	0.337
$\text{H}^{+1}_{(\text{aq})} + 2\text{e}^{-} \rightarrow \text{H}_{2(\text{g})}$	0 (standard)
$\text{Pb}^{+2}_{(\text{aq})} + 2\text{e}^{-} \rightarrow \text{Pb}^0_{(\text{s})}$	-0.126
$\text{Fe}^{+2}_{(\text{aq})} + 2\text{e}^{-} \rightarrow \text{Fe}^0_{(\text{s})}$	-0.441
$\text{Zn}^{+2}_{(\text{aq})} + 2\text{e}^{-} \rightarrow \text{Zn}^0_{(\text{s})}$	-0.763

Copper ion (Cu^{+2}) has a higher standard reduction potential than zinc ion (Zn^{+2}), meaning that, under identical conditions, more energy is released by reducing one mole of Cu^{+2} ion to Cu^0 metal than is released by reducing one mole of Zn^{+2} ion to Zn^0 metal. Another way to think about this is to imagine that the copper ion 'wants' to gain electrons more than the zinc ion does. Conversely, zinc metal 'wants' to *lose* electrons more than the copper metal does. Therefore, transfer of two electrons from zinc metal to Cu^{2+} is a thermodynamically downhill process, whereas the reverse process - transfer of two electrons from copper metal to Zn^{2+} - is thermodynamically uphill.

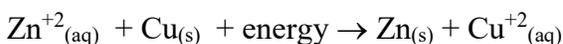


Let's now extend the idea of redox reactions to the context of metabolism in living things. When we 'burn' glucose for energy, we transfer (by a series of enzyme-catalyzed reactions) electrons from glucose to molecular oxygen (O_2), oxidizing the six carbon molecules in glucose to carbon dioxide and at the same time reducing the oxygen atoms in O_2 to water. The overall chemical equation is:



The transfer of electrons from glucose to O_2 is a thermodynamically downhill, energy-releasing process, just like the transfer of electrons from zinc metal to copper ion. And while you could have used the energy released by the zinc/copper redox reaction to light a small light bulb, your cells use the energy released by the glucose/oxygen redox process to carry out a wide variety of energy-requiring activities, such as walking to your organic chemistry lecture.

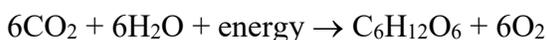
In your general chemistry copper/zinc experiment, was it possible to reverse the reaction so that it runs in the uphill direction - in other words, to oxidize copper and reduce zinc?



Just ask yourself the question: is it possible to get water to flow uphill? Of course it is - but only if you supply a pump and some energy!

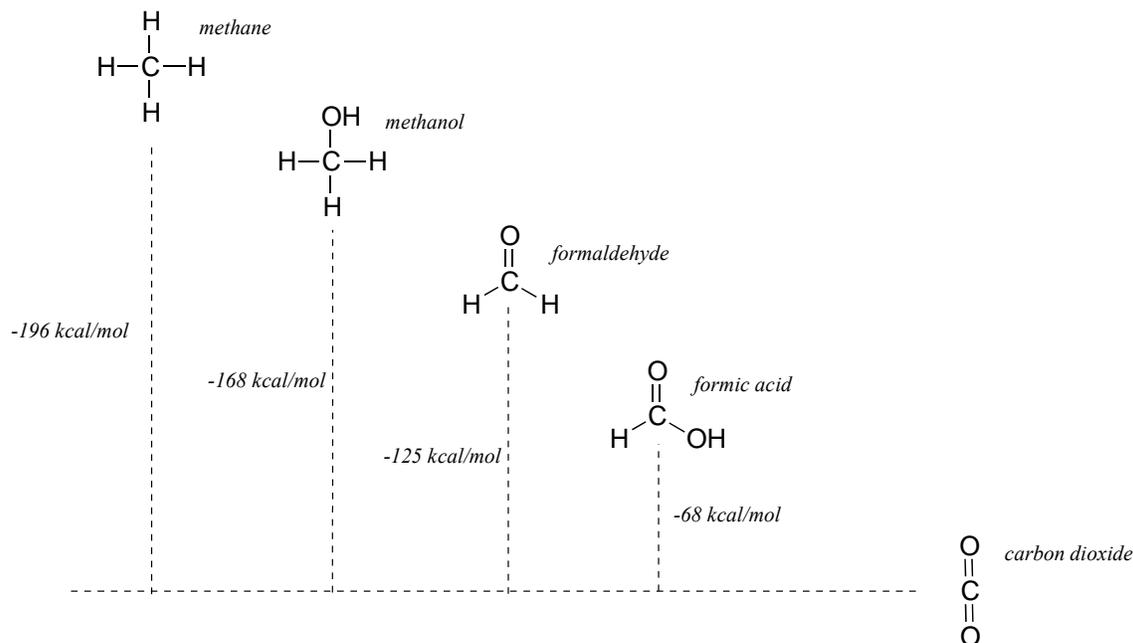
The same idea applies to 'pumping' electrons uphill in your copper-zinc electrochemical cell: all you need to do is to provide some energy in the form of an external electrical current in order to pump the electron flow in the uphill direction. You are recharging your battery.

Thinking again in a biochemical context: plants are able, by a process called photosynthesis, to reduce carbon dioxide and oxidize water to form glucose and molecular oxygen: essentially recharging the ecosystem's biochemical battery using energy from the sun.

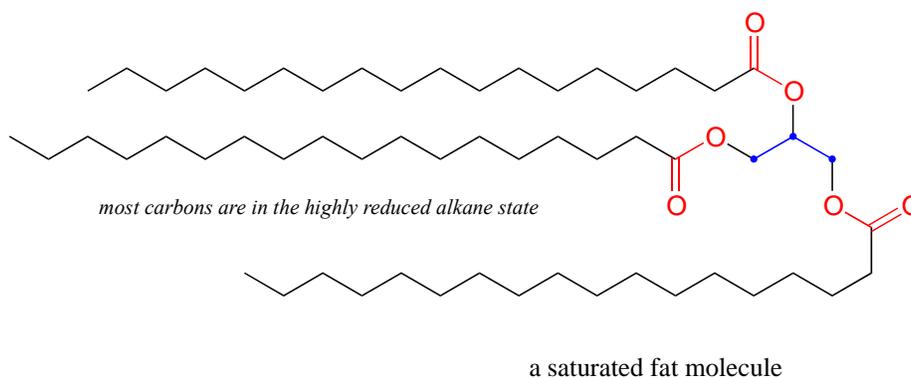


On a global scale, oxidation of the carbons in glucose to CO_2 by non-photosynthetic organisms (like people) and the subsequent reductive synthesis of glucose from CO_2 by plants is what ecologists refer to as the 'carbon cycle'.

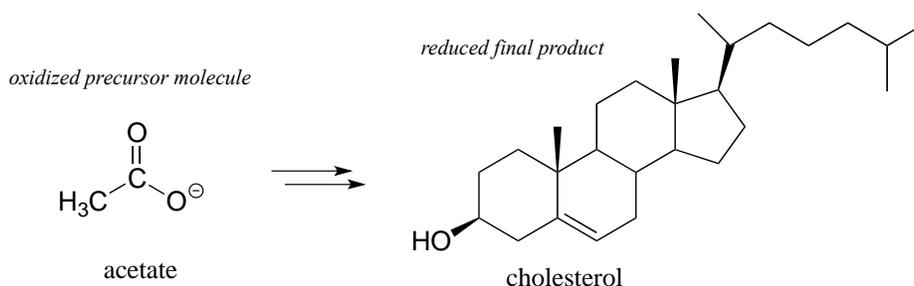
In general the more reduced an organic molecule is, the more energy is released when it is oxidized to CO_2 . Going back to our single-carbon examples, we see that methane, the most reduced compound, releases the most energy when oxidized to carbon dioxide, while formic acid releases the least:

energy of oxidation to CO₂:

A lipid (fat) molecule, where most of the carbons are in the highly reduced alkane state, contains more energy per gram than glucose, where five of the six carbons are in the more oxidized alcohol state (look again at the glucose structure we saw just a couple of pages back).



After we break down and oxidize sugar and fat molecules to obtain energy, we use that energy to build large, complex molecules (like cholesterol, or DNA) out of small, simple precursors. Many biosynthetic pathways are *reductive*: the carbons in the large biomolecule products are in a reduced state compared to the small precursors. Look at the structure of cholesterol compared to that of acetate, the precursor molecule from which all of its carbon atoms are derived - you can see that cholesterol is overall a more reduced molecule.



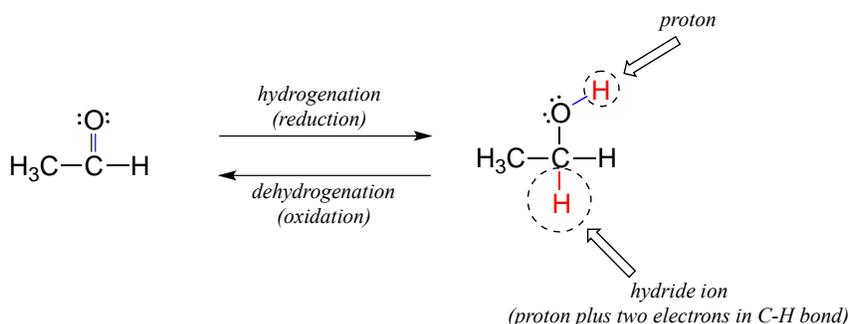
While we are focusing here on the mechanistic details of the individual organic redox reactions involved in metabolism, if you take a course in biochemistry you will learn much more about the bigger picture of how all of these reactions fit together in living systems.

Section 15.3: Hydrogenation of carbonyl and imine groups

Next, we'll go on to look at the actual chemical mechanisms involved in the enzyme-catalyzed oxidation and reduction of biomolecules.

15.3A: An overview of hydrogenation and dehydrogenation

Many of the redox reactions that you will encounter when studying the central metabolic pathways are classified as **hydrogenation** or **dehydrogenation** reactions. Hydrogenation is simply the net addition of a hydrogen (H_2) molecule to a compound, in the form of a **hydride ion** (H^- , a proton plus a pair of electrons) and a proton. **Hydrogenation corresponds to reduction**. Dehydrogenation reactions are the reverse process: loss of a hydride and a proton. **Dehydrogenation corresponds to oxidation**.



Hydrogenation and dehydrogenation reactions can also be called **hydride transfer** reactions, because a hydride ion is transferred from the molecule being oxidized to the one being reduced. In the next few sections, we will learn about two important classes of

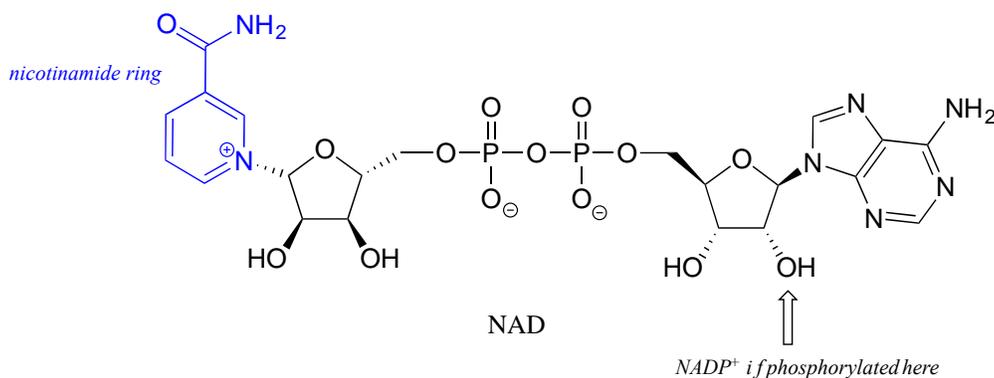
coenzyme molecules that serve **hydride ion acceptors** (oxidizing agents) and **hydride ion donors** (reducing agents) in biochemical redox reactions.

Be careful not to confuse the terms *hydrogenation* and *dehydrogenation* with *hydration* and *dehydration* - the latter terms refer to the gain or loss of *water*, while the former terms refer to the gain or loss of *hydrogen*.

Many mechanistic patterns that we have already learned about in previous chapters will come into play again in this discussion, with the only variation being that here, a *hydride ion* will act as a nucleophile (in the hydrogenation direction) or as a leaving group (in the dehydrogenation direction). The key to understanding these reactions will be to understand how a hydride can act as a nucleophile or leaving group.

15.3B: Nicotinamide adenine dinucleotide - a hydride transfer coenzyme

Although we are talking here about hydrides acting as nucleophiles and leaving groups, you already know that *literal* hydride ions are far too unstable to exist as discrete intermediates in the organic reactions of living cells (the pKa of H₂, the conjugate acid of hydride, is about 35: a *very* weak acid, meaning hydride is a *very* strong base and not a reasonable species to propose for a biochemical reaction). As was alluded to earlier, biochemical hydrogenation/dehydrogenation steps require the participation of a specialized hydride transfer coenzyme. The most important of these is a molecule called **nicotinamide adenine dinucleotide**. The full structure of the oxidized form of this coenzyme, abbreviated **NAD⁺**, is shown below, with the active nicotinamide group colored blue. Because the redox chemistry occurs specifically at the nicotinamide ring (in blue in the figure below), typically the rest of the molecule is simply designated as an 'R' group.

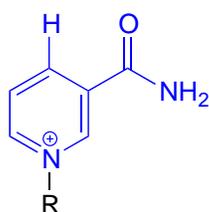


If the hydroxyl group indicated by the arrow is phosphorylated, the coenzyme is called **NADP⁺**. The phosphate is located far from the nicotinamide ring and does not participate

directly in the hydride transfer function of the cofactor. It is, however, important in a larger metabolic context: as a general rule, redox enzymes involved in catabolism (the breakdown of large molecules) typically use the non-phosphorylated coenzyme, while those involved in anabolism (biosynthesis of large molecules from small precursors) use the phosphorylated coenzyme.

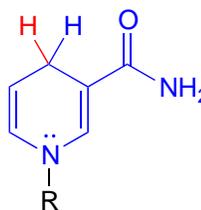
NAD^+ and NADP^+ both function in biochemical redox reactions as **hydride acceptors**: that is, as oxidizing agents. The reduced forms of the coenzyme, abbreviated NADH and NADPH , serve as **hydride donors**: that is, as reducing agents.

oxidized form
hydride acceptor
oxidizing agent



NAD(P)^+

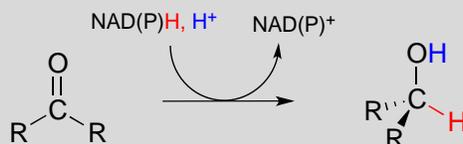
reduced form
hydride donor
reducing agent



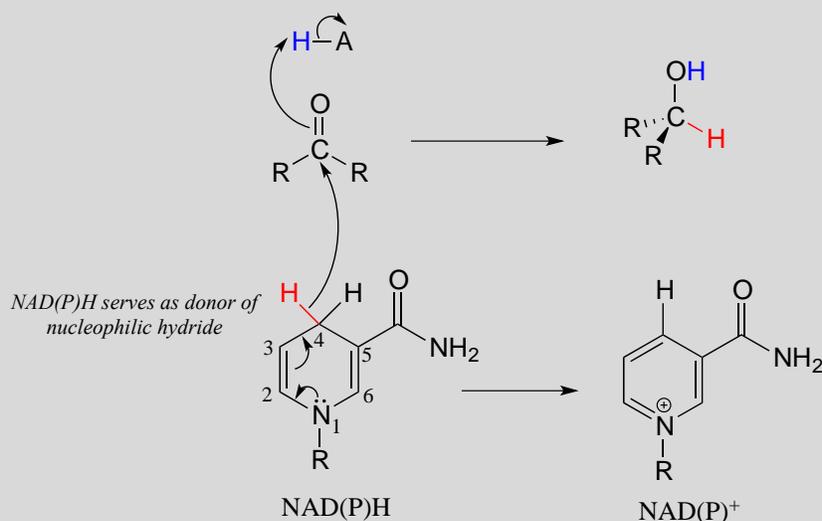
NAD(P)H

To understand how the nicotinamide coenzymes function in hydride transfer, let's look at a general picture of a reversible, redox conversion from a ketone to a secondary alcohol. Mechanistically, the reaction we are about to see can be described as a nucleophilic addition to a carbonyl - a mechanism type we studied in chapter 10 - with the twist that the nucleophilic species is a hydride ion. At the beginning of the reaction cycle, both the ketone substrate and the NADH cofactor are bound in the enzyme's active site, and carbon #4 of the nicotinamide ring is positioned very close to the carbonyl carbon of the ketone.

NAD(P)H -dependent hydrogenation (reduction) of a ketone



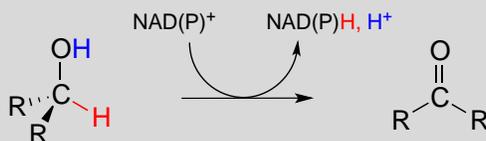
Mechanism:



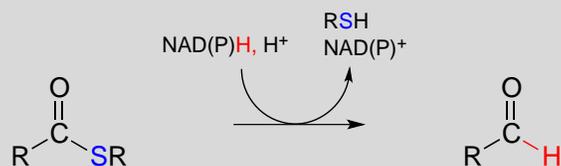
As an enzymatic group transfers a proton to the ketone oxygen, the carbonyl carbon loses electron density and becomes more electrophilic, and is attacked by a hydride from NADH. Because carbon #4 of NADH is bound in such close proximity to the electrophile, this step can occur *without generating a free hydride ion intermediate* – the two hydride electrons can be pictured as *shifting* from one carbon to another. Note the products of this reaction: the ketone (which accepted a hydride and a proton) has been reduced to an alcohol, and the NADH cofactor (which donated a hydride) has been oxidized to NAD^+ .

The dehydrogenation of an alcohol by NAD^+ is simply the reverse of a ketone hydrogenation:

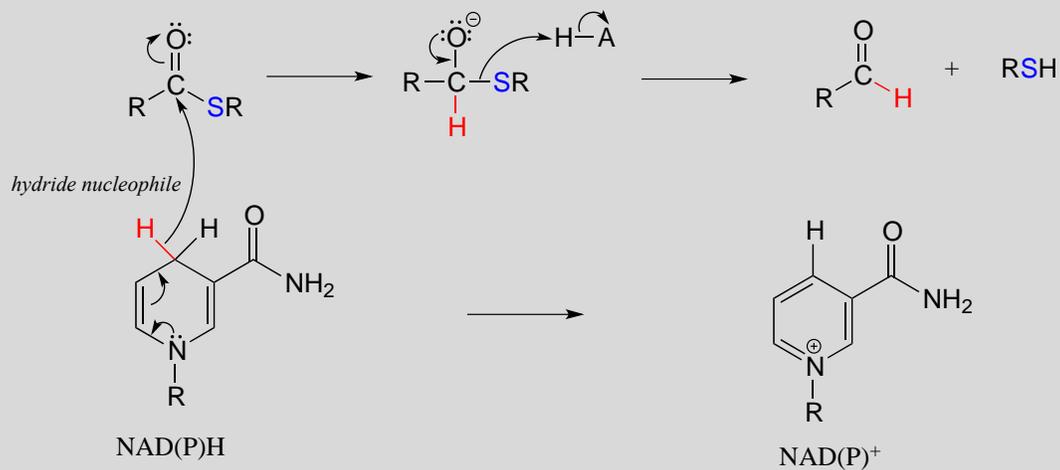
NAD(P)^+ -dependent dehydrogenation (oxidation) of an alcohol



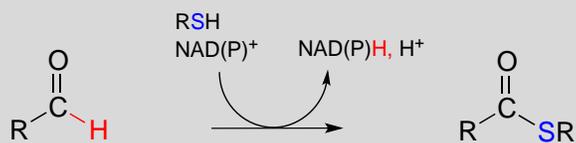
NAD(P)H-dependent hydrogenation (reduction) of a thioester to an aldehyde:



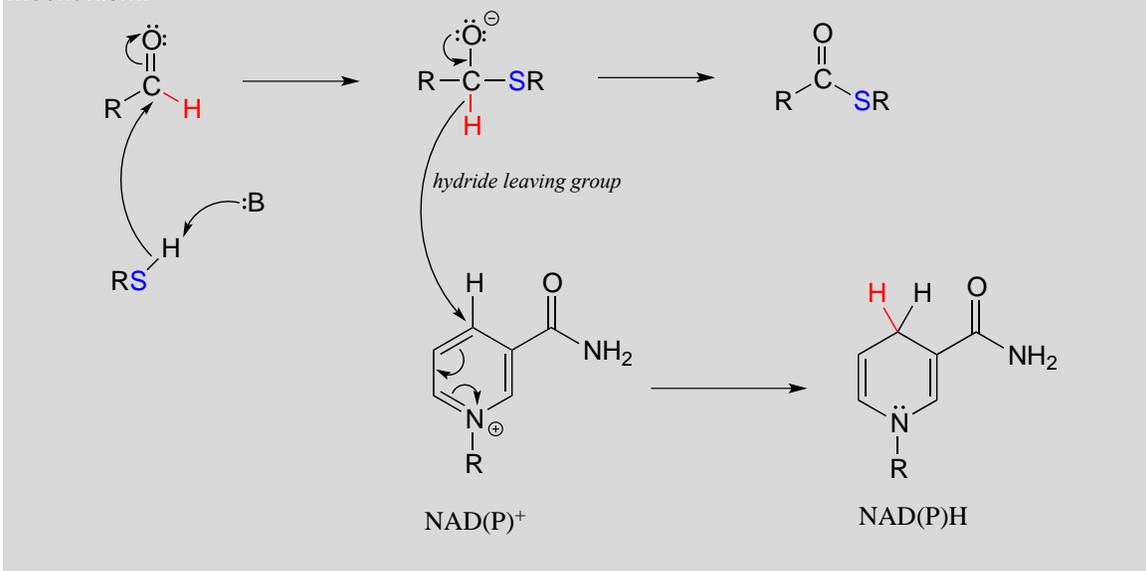
Mechanism:



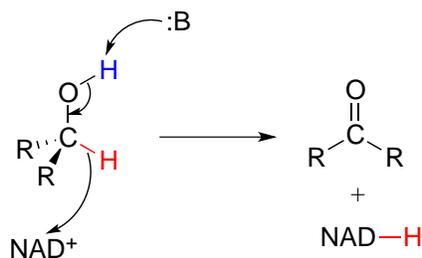
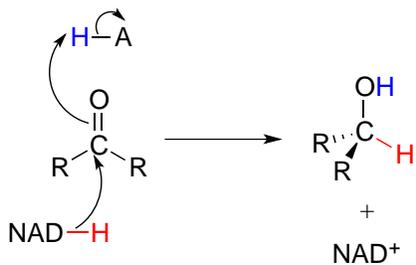
NAD(P)⁺-dependent dehydrogenation (oxidation) of an aldehyde to a thioester:



Mechanism:



To simplify figures, hydrogenation and dehydrogenation reactions are often drawn with the role of the coenzyme abbreviated:

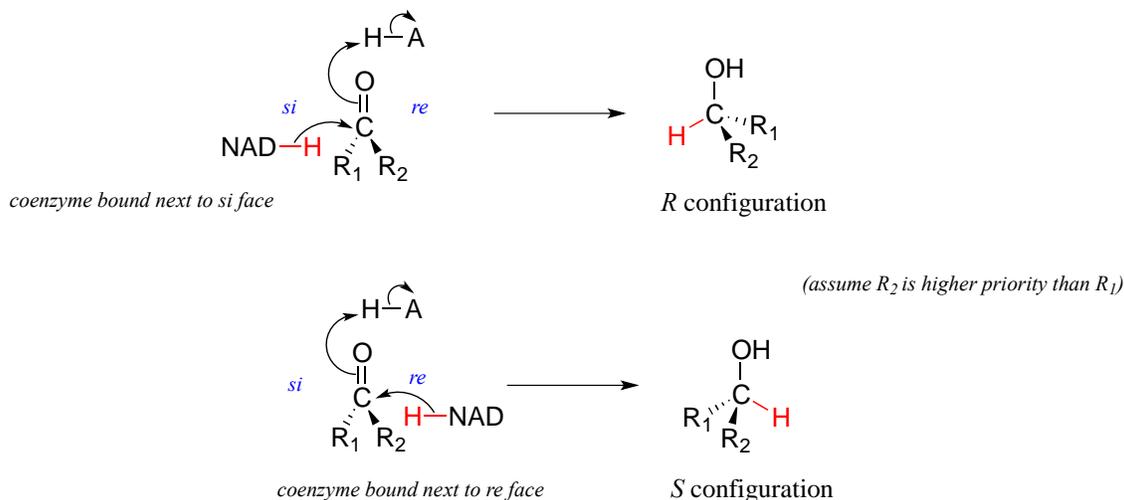


However, it is very important to make sure that you can remember and draw out the full mechanism, including the role of the coenzyme, in these types of reactions.

Caution! A very common error made by students learning how to draw biochemical redox mechanisms is to incorrectly show nicotinamide coenzymes acting as acids or bases. Remember: NADH and NADPH are *hydride* donors, **NOT** proton donors. NAD⁺ and NADP⁺ are *hydride* acceptors, **NOT** proton acceptors.

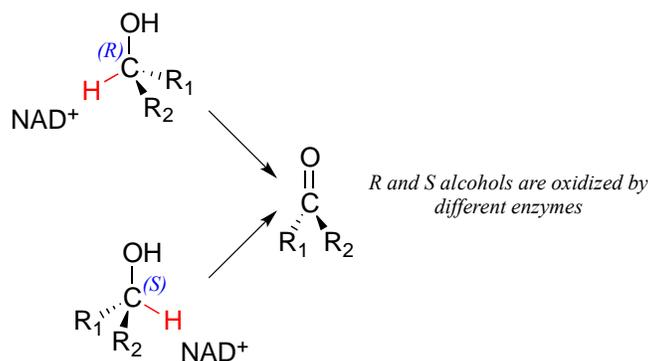
15.3C: Stereochemistry of ketone hydrogenation

It should not surprise you that the stereochemical outcomes of enzymatic hydrogenation / dehydrogenation steps are very specific. Consider the hydrogenation of an asymmetric ketone: In the hydrogenation direction, attack by the hydride can occur from either the *re* or the *si* face of an asymmetric ketone (see section 10.1C), leading specifically to the *S* or *R* alcohol.

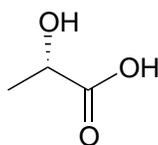


The stereochemical configuration of the product depends on which side of the ketone substrate the NAD(P)H coenzyme is bound in the active site. Any given enzyme will catalyze its reaction with *one* of these two stereochemical outcomes, not both.

Stereochemical considerations apply in the dehydrogenase direction as well: in general, enzymes specifically catalyze the oxidation of either an *R* or *S* alcohol, but not both.



Exercise 15.6: During an intense workout, lactic acid forms in muscle tissue as the result of enzymatic reduction of a ketone group in the precursor molecule (EC 1.1.1.27). It is the lactate that you can blame for the sore muscles you feel the day after a workout.



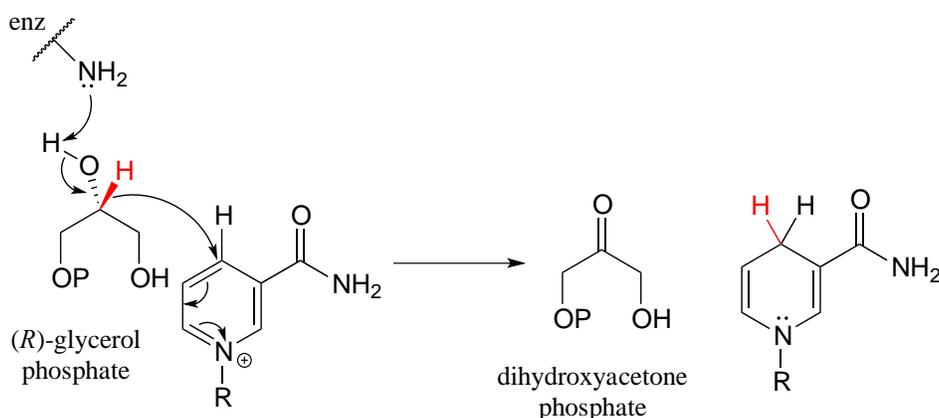
lactic acid

- a) Draw the structure of the starting ketone in this reaction.
- b) Which face of the ketone is the coenzyme positioned next to in the active site of the enzyme?

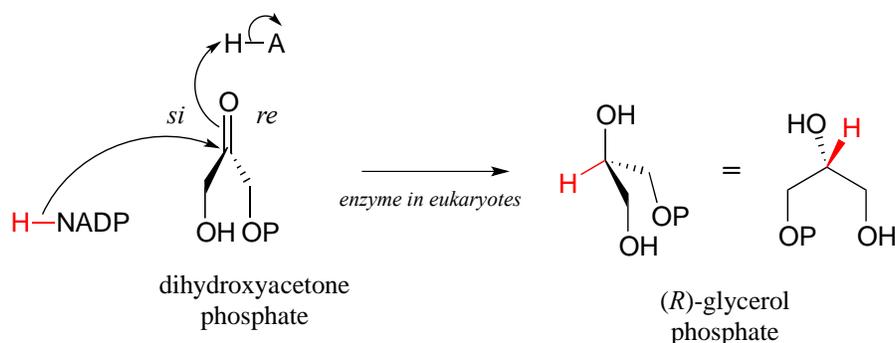
15.3D: Examples of biochemical carbonyl/imine hydrogenation

Now that we have covered the basics, let's look at some real examples of hydrogenation and dehydrogenation reactions.

Glycerol phosphate dehydrogenase (EC 1.1.1.8) catalyzes one of the final chemical steps in the breakdown of fat molecules. The enzyme specifically oxidizes (*R*)-glycerol phosphate to dihydroxyacetone phosphate. (*S*)-glycerol phosphate is *not* a substrate for this enzyme.

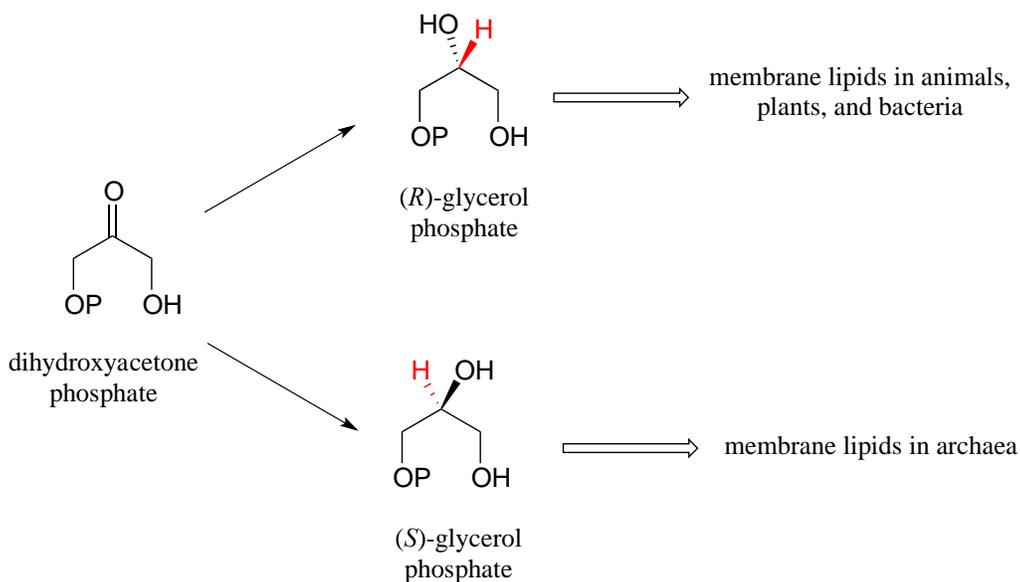


The reverse reaction (catalyzed by the same enzyme) converts dihydroxyacetone phosphate to (*R*)-glycerol phosphate, which serves as a starting point for the biosynthesis of membrane lipid molecules (see section 1.3A).

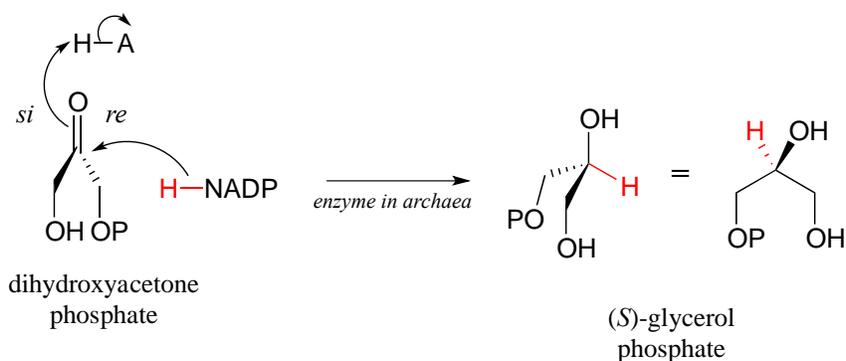


Exercise 15.7: X-ray crystallography experiments reveal that in the active site of glycerol phosphate dehydrogenase, a zinc cation (Zn^{2+}) is coordinated to the oxygen atom of the carbonyl/alcohol group. How does this contribute to catalysis of the reaction?

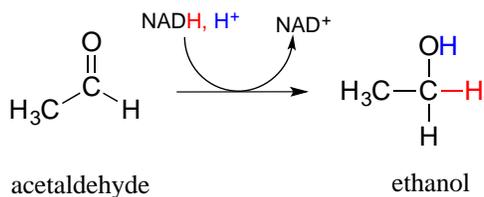
While the cell membranes of animals, plants, and bacteria are made from lipids with the *R* stereochemistry exclusively, archaeal microbes (the so-called 'third kingdom of life') are distinguished in part by the *S* stereochemistry of their membranes.



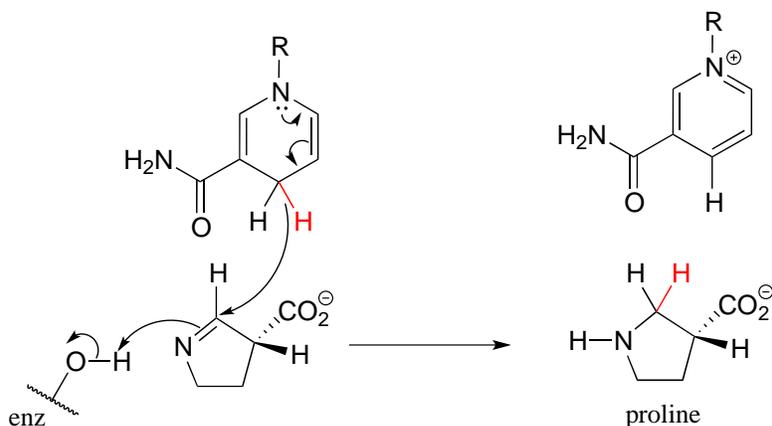
Archaea have an enzyme that catalyzes hydrogenation of dihydroxyacetone with the opposite stereochemistry compared to the analogous enzyme in bacteria and eukaryotes. This archaeal enzyme was identified and isolated in 1997.



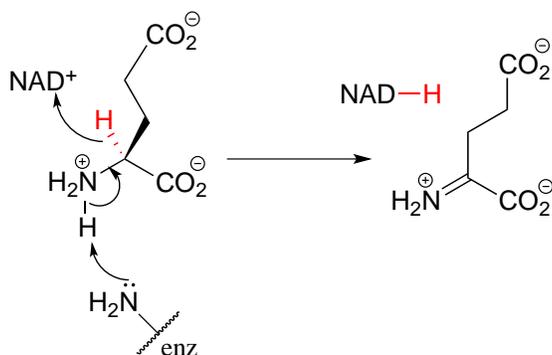
In a reaction that is relevant to people who enjoy the occasional 'adult beverage', an NADH-dependent enzyme (EC 1.1.1.1) in brewer's yeast produces ethanol by reducing acetaldehyde. This is the final step in the process by which yeast ferment glucose to ethanol.



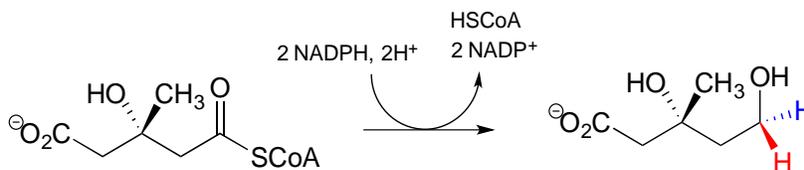
The reaction below, which is the final step in the biosynthesis of proline (EC 1.5.1.2), is an example of an enzymatic reduction of an imine to an amine.



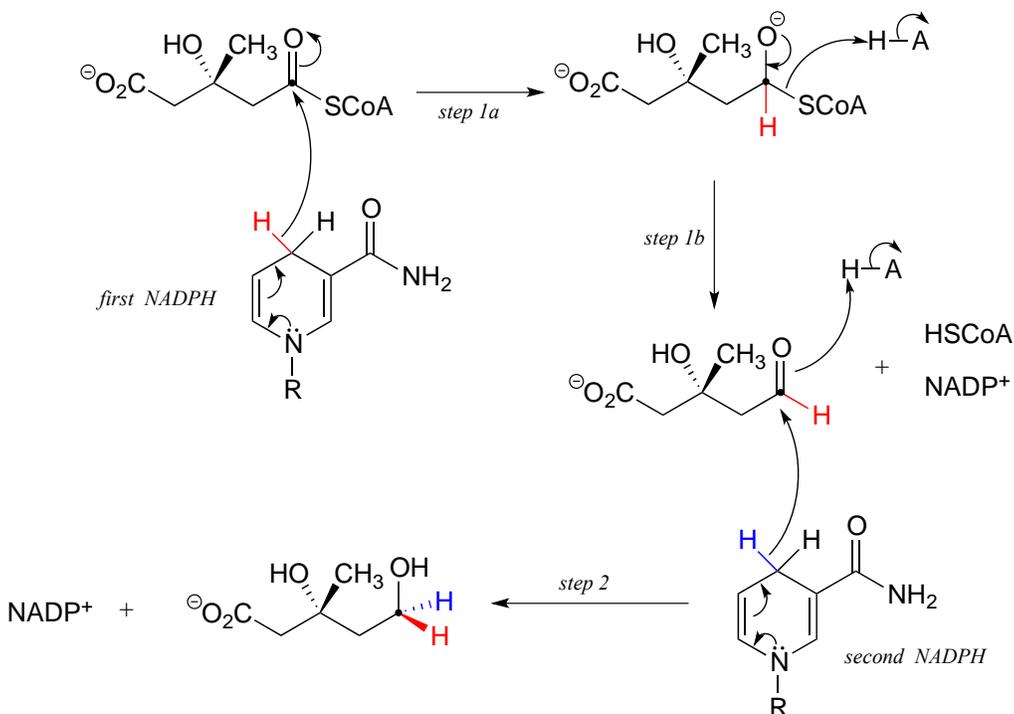
This step in the breakdown of the amino acids glutamate (EC 1.4.1.2) provides an example of the oxidation of an amine to an imine:



The 'double reduction' reaction below (EC 1.1.1.34) is part of the isoprenoid biosynthetic pathway, which eventually leads to cholesterol in humans.



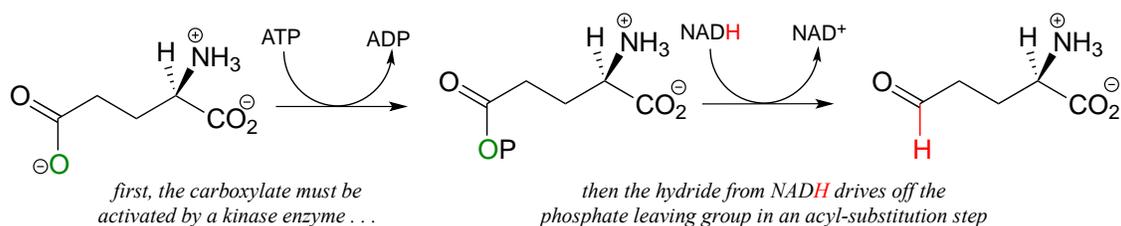
In this reaction, a thioester is first reduced to an aldehyde in steps 1a and 1b:



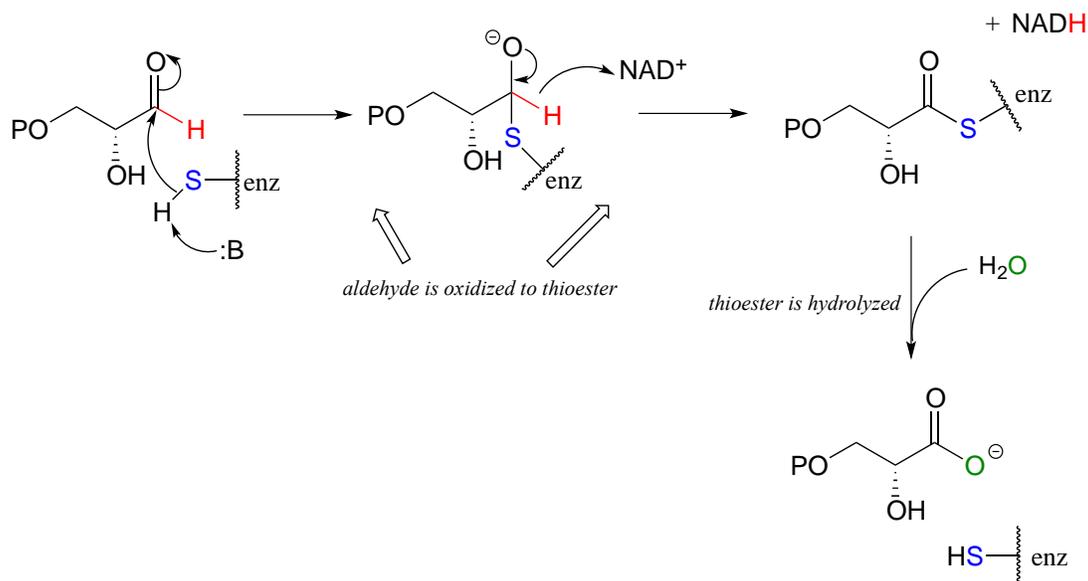
Then in step 2, the aldehyde is in turn reduced by the same enzyme (and a second NADPH that enters the active site) to a primary alcohol. This enzyme is inhibited by

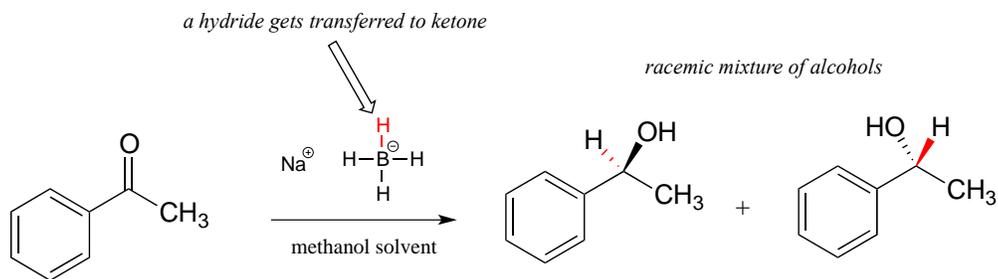
atorvastatin and other members of the statin family of cholesterol-lowering drugs. Atorvastatin, marketed under the trade name Lipitor by Pfizer, is one of the all-time best-selling prescription medications.

Recall from chapter 11 that carboxylates are not reactive in acyl substitution steps, so it follows that they cannot be *directly* reduced to aldehydes by an enzyme in the same way that thioesters can. However, a carboxylate can be converted to its 'activated' acyl phosphate form (section 11.4), which can then be hydrogenated. An example of this is found in a two-reaction sequence found in amino acid metabolism (EC 2.7.2.11; EC1.2.1.41).



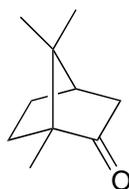
Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), a key enzyme in the glycolysis pathway, provides an example of the oxidation of an aldehyde to a thioester, in this case a thioester linkage between the substrate and a cysteine residue in the enzyme's active site. In the second phase of the reaction, the thioester intermediate is hydrolyzed to free the carboxylate product.





Synthetic organic chemists have at their disposal a wide range of other reducing and oxidizing reagents with varying specificities and properties, many of which you will learn about if you take a course in laboratory synthesis.

Exercise 15.10: Camphor can be easily reduced by sodium borohydride. However, the mixture of stereoisomeric alcohols that results is not 50:50.

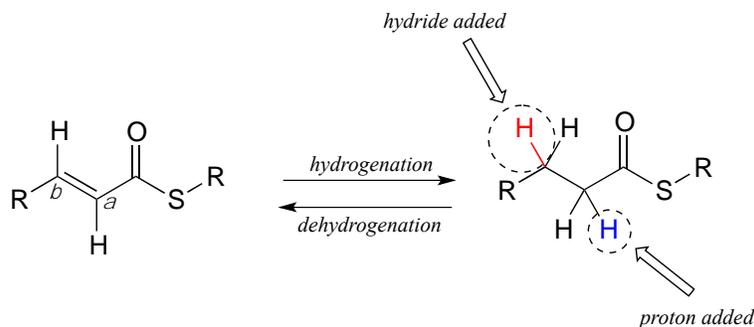


camphor

- Draw the two stereoisomers of the alcohol products of this reaction, and explain why they are not formed in a 50:50 ratio.
- Which analytical technique - $^1\text{H-NMR}$, IR, UV, or MS - could best be used to determine the ratio of the stereoisomers in the product mix? Describe how this analysis could be accomplished.

Section 15.4: Hydrogenation of alkenes and dehydrogenation of alkanes

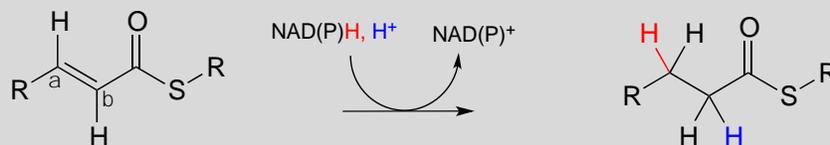
We turn next to reactions in which a hydrogen molecule is added to the double bond of an alkene, forming an alkane - and the reverse, in which H_2 is eliminated from an alkane to form an alkene. Many biochemical reactions of this type involve α,β -unsaturated thioesters.



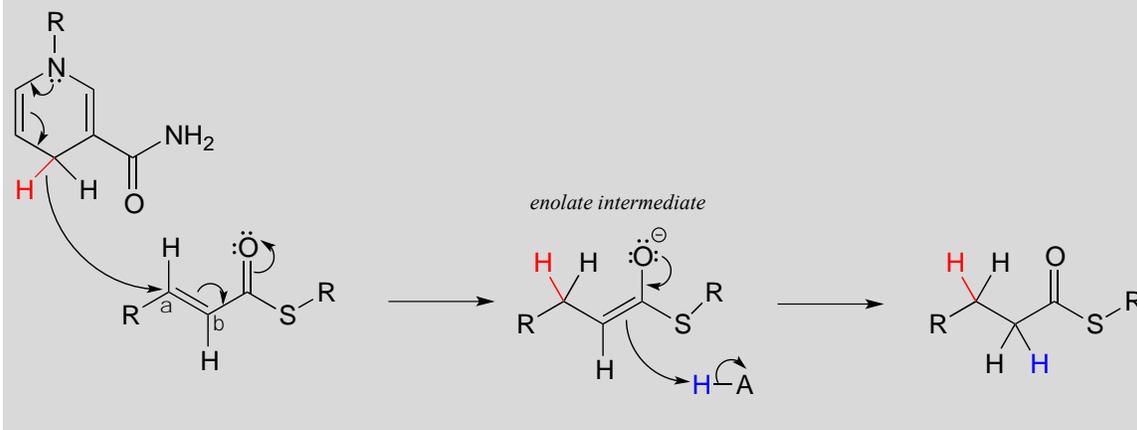
15.4A: Alkene hydrogenation

In the cell, alkene hydrogenation most often occurs at the α and β position relative to a carbonyl. This type of alkene hydrogenation is essentially a conjugate addition (section 11.4) of hydrogen, with a hydride ion (often from NAD(P)H) acting as the nucleophile in the first step.

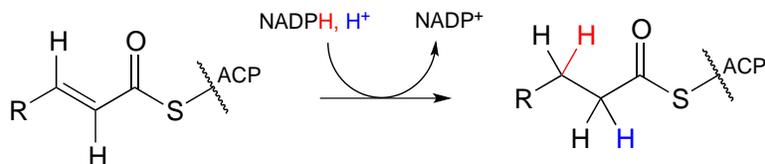
NAD(P)H-dependent hydrogenation (reduction) of an α,β -conjugated alkene:



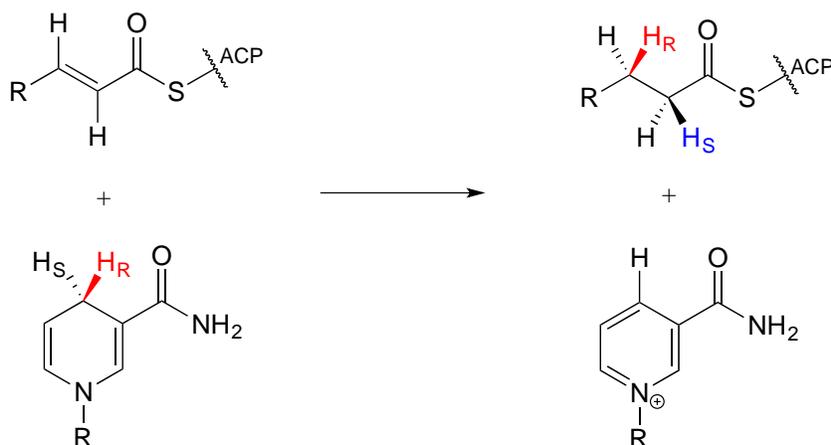
Mechanism:



As part of the fatty acid synthesis pathway, a double bond between the α and β carbons of a fatty acid is reduced to a single bond by hydrogenation (EC 1.3.1.10). The fatty acid is attached to an acyl-carrier protein via a thioester linkage (section 11.5A).



It can be easy to forget but important to remember that there is a lot of stereospecificity inherent in biochemical reactions, including this one - even though no chiral centers are involved. First, notice that the substrate contains a *trans* (*E*) alkene. Next, let's add some more information about *prochirality*:

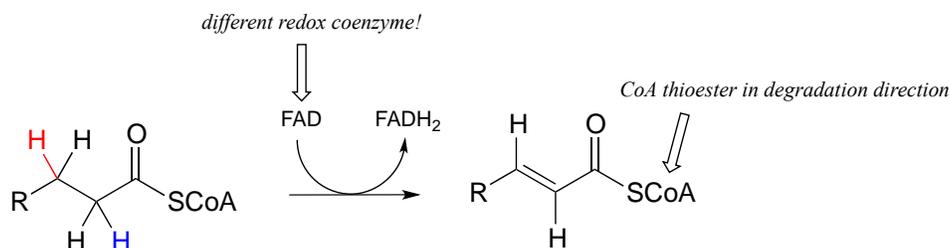


Notice that in this particular reaction it is specifically the *pro-R* hydride on NADPH that is delivered to the substrate. Also notice that both the hydride and proton are added to the *re* faces of the α and β carbons of the substrate, and become the *pro-R* and *pro-S* hydrogen atoms, respectively, on the product. This level of stereospecificity, you should recall from previous discussions, stems from the highly precise positioning of substrate and cofactor within the active site of the enzyme.

Other hydrogenase enzymes are known to deliver the *pro-S* hydride of NADH or NADPH to their substrate, and there are many examples of biochemical conjugate addition reactions in which the nucleophile and proton are added from *opposite* sides. Always keep in mind that stereochemistry is a key element in the amazing diversity of biological organic reactions.

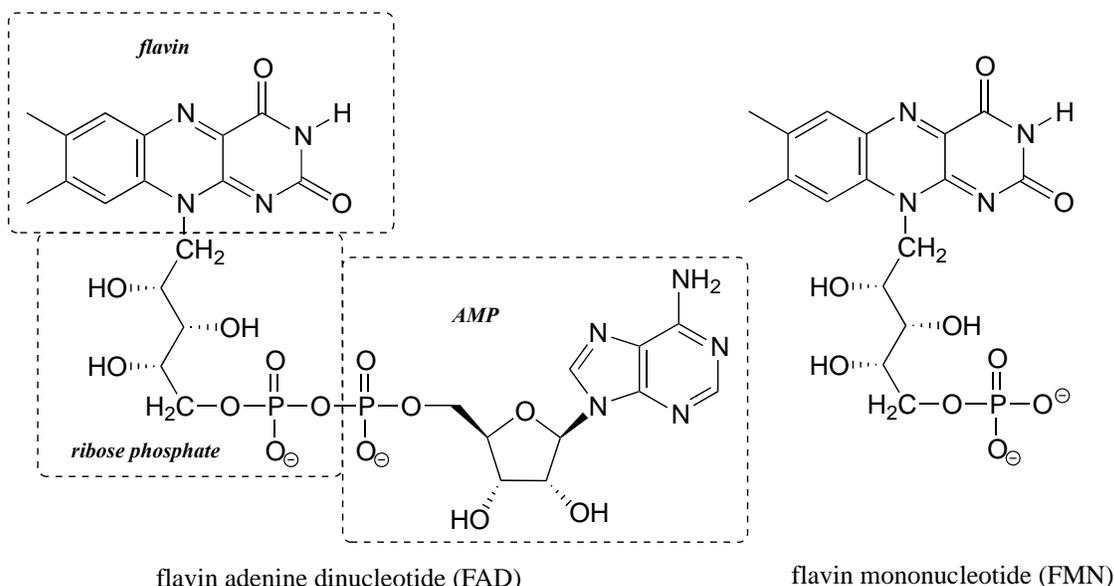
15.4B: Flavin-dependent alkane dehydrogenation

Next, let's consider an alkane dehydrogenation reaction (EC 1.3.99.3) in the fatty acid degradation pathway. Here, a double bond is introduced between the α and β carbons, with concurrent loss of a hydride ion and a proton.



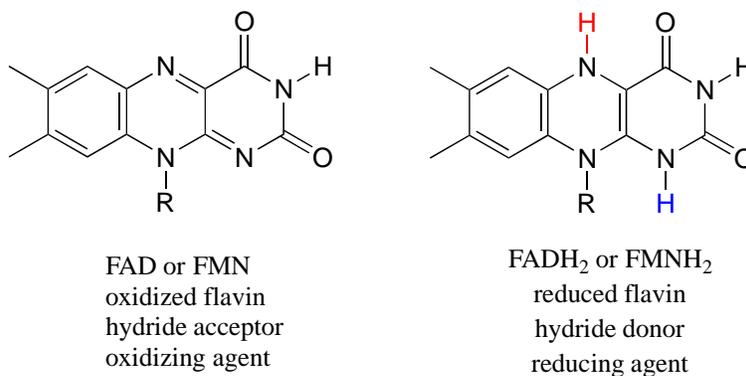
This reaction is clearly *not* the reverse of the hydrogenation reaction we just saw from fatty acid biosynthesis. First of all, you should notice that the thioester linkage is to coenzyme A rather than acyl carrier protein (ACP). More importantly to this discussion, while the hydride donor in the biosynthetic hydrogenation reaction is NADPH, the relevant coenzyme in the catabolic direction is *not* NAD⁺ or NADP⁺ - rather, it is a **flavin coenzyme**.

Flavin adenine dinucleotide (FAD) is composed of three components: the three-ring flavin system, ribose phosphate, and AMP. An alternate form, which is missing the AMP component, is called **flavin mononucleotide (FMN)**.



The reactive part of the coenzyme is the flavin group, so usually the rest of the molecule is abbreviated with 'R'.

FAD and FMN are the oxidized form of flavin. The reduced (hydrogenated) forms of these cofactors are abbreviated FADH₂ and FMNH₂.

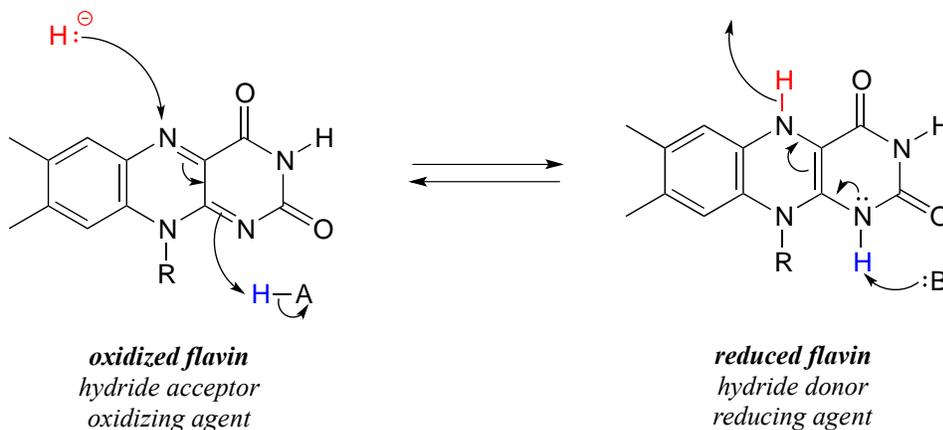


The flavin coenzymes are synthesized in humans from riboflavin (vitamin B₂), which we obtain from our diet (the structure of riboflavin is the same as that of FMN, except that riboflavin lacks the phosphate group). Notice the extended conjugated π system in the three fused rings: the flavin system absorbs light in the visible wavelengths and has a distinctive deep yellow color - it is riboflavin, and to some extent FAD and FMN, that give urine its color.

Like the nicotinamide coenzymes, flavin serves as a hydride donor or acceptor. FAD and FMN are able to accept a hydride ion (and a proton), and FADH₂ and FMNH₂ in turn can serve as hydride donors in hydrogenation reactions.

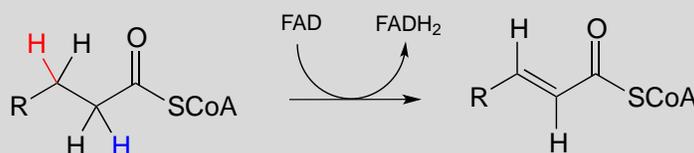
hydride delivered from biomolecule being oxidized

hydride delivered to biomolecule being reduced

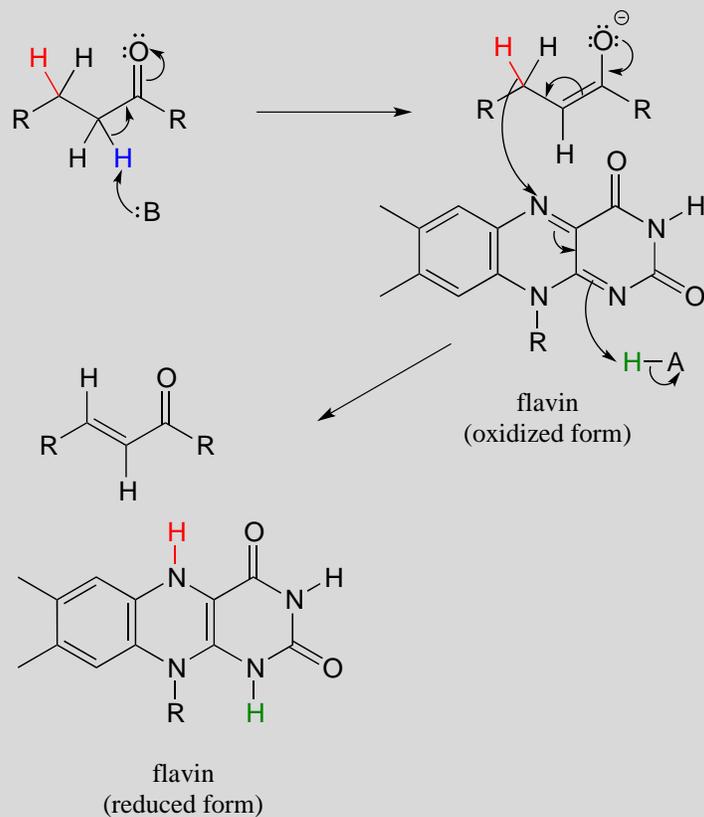


Below is a general mechanism for the dehydration of an alkene at the α,β position - notice that it is mechanistically an E1cb elimination of H₂.

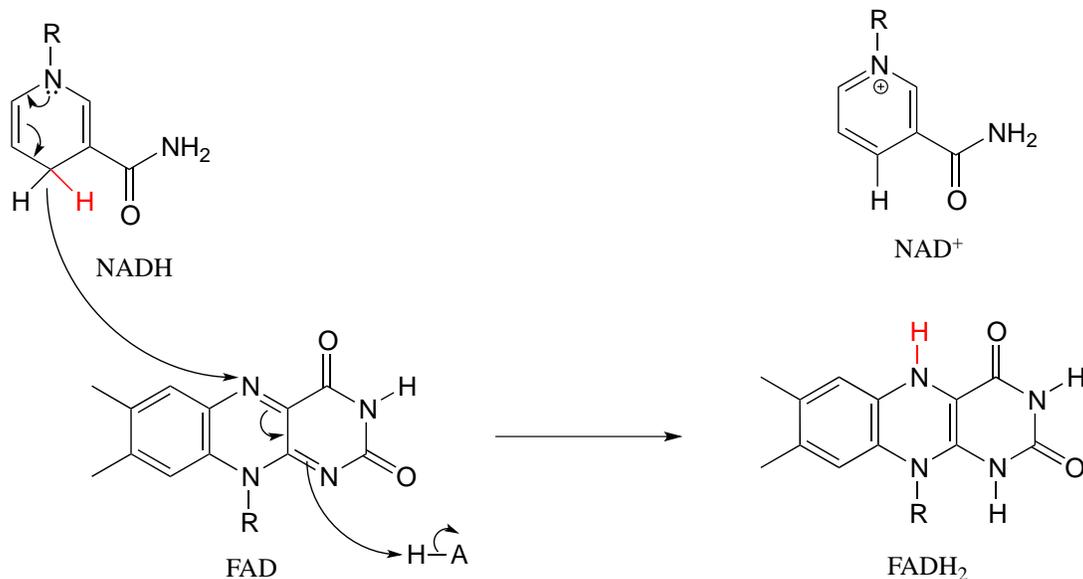
Flavin-dependent α,β dehydrogenation (oxidation) of an alkane:



Mechanism:

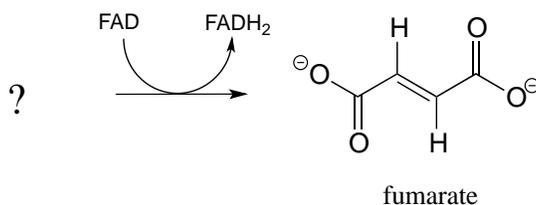


In many enzymatic reactions in which FADH₂ acts as the reducing agent, the reaction cycle is completed when FAD, rather than being released from the active site, is recycled back to FADH₂ with the concomitant oxidation of NADH.



Hydride ion transfer with flavin or nicotinamide coenzymes is a *two electron* redox process. However, unlike the nicotinamide cofactors, flavins are also able to function in *single* electron transfer (radical) mechanisms. We will come back to this idea briefly in the chapter 16.

Exercise 15.11: Fumarate is formed in an alkane dehydrogenation reaction (EC 1.3.5.1) which is part of the citric acid cycle:



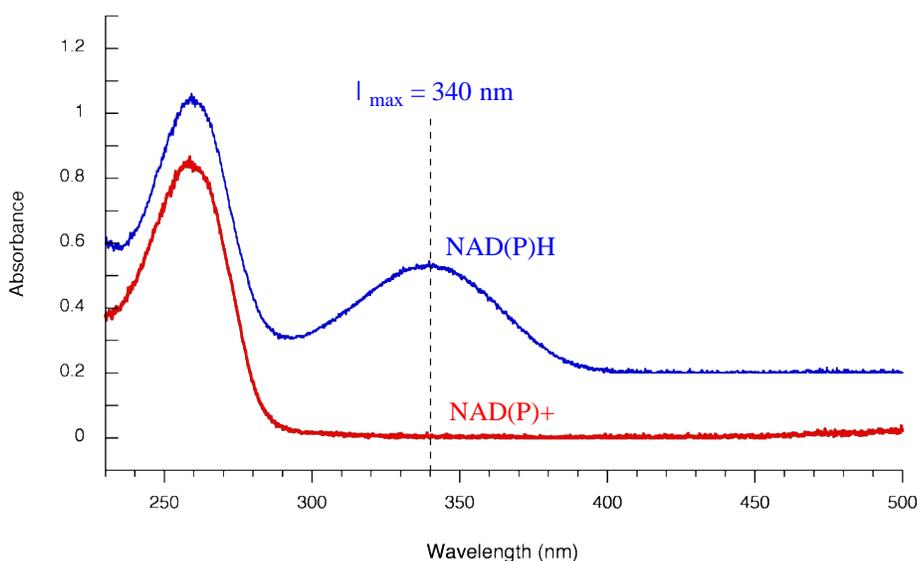
- Predict the structure of the starting substrate in this reaction
- Draw the structure of the enolate intermediate

Exercise 15.12: Reduced flavin can serve as the hydride *donor* in some hydrogenation reactions. Degradation of the RNA base uracil begins with hydrogenation of a conjugated alkene group by a flavin-dependent hydrogenase enzyme (EC 1.3.1.2). Predict the product of this step, and draw curved arrows for the first mechanistic step.

Section 15.5: Monitoring hydrogenation and dehydrogenation reactions by UV spectroscopy

In order to study any enzyme-catalyzed reaction, a researcher must have available some sort of test, or **assay**, in order to observe and measure the reaction's progress and measure its rate. In many cases, an assay simply involves running the reaction for a specified length of time, then isolating and quantifying the product using a separation technique such as high performance liquid chromatography (HPLC) or gas chromatography (GC). This type of assay can be extremely time-consuming, however, so it is to the researcher's great advantage if a more convenient assay can be found.

Redox reactions in which a nicotinamide coenzyme participates as a hydride donor or acceptor are generally quite convenient to assay. In fact, the progress of these reactions can usually be observed in real time, meaning that the researcher doesn't need to stop the reaction in order to see how far it has progressed. NADPH and NADH have distinctive $n-\pi^*$ UV absorbance bands centered at 340 nm, with a molar absorptivity of $6290 \text{ M}^{-1} \text{ cm}^{-1}$ (section 4.4). The oxidized coenzymes NADP^+ and NAD^+ do not absorb at this wavelength.

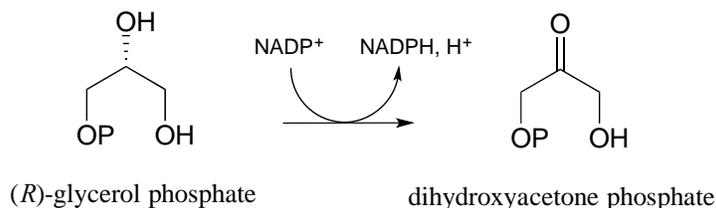


Therefore, the course of a hydrogenation reaction, in which NAD(P)H is converted to NAD(P)^+ , can be observed in real time if it is run in a quartz cuvette in a UV spectrometer. By observing the decrease in absorbance at 340 nm, the researcher can calculate how much NAD(P)H has been oxidized to NAD(P)^+ at any given time point, and this number is the molar equivalent of the amount of organic substrate that has been reduced:



Likewise, a NAD^+ -dependent dehydrogenase reaction can be followed in real time by monitoring the *increase* in absorbance at 340 nm as NAD^+ is converted to NADH.

Exercise 15.13: You are observing the progress of the (*R*)-glycerol phosphate dehydrogenase reaction shown in the figure below.

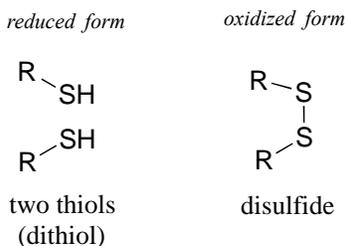


You run the reaction in a quartz cuvette (path length 1 cm) in a total solution volume of 1 mL. You start with 200 μM substrate and 100 μM NADP^+ in solution, zero the UV spectrophotometer, then add the enzyme to start the reaction. After 5 minutes, the A_{340} reading has climbed from 0.000 to 0.096. At this time point:

- How many moles of substrate have been oxidized?
- What is the solution concentration of NADP^+ ?
- The enzyme has a mass of 25 kilodaltons (25,000 g/mol). You added 5 μL of a 2 ng/ μL solution of pure enzyme to start the reaction. How many reactions does each enzyme molecule catalyze, on average, per second? (This number is referred to by biochemists as the 'turnover number').

Section 15.6: Redox reactions of thiols and disulfides

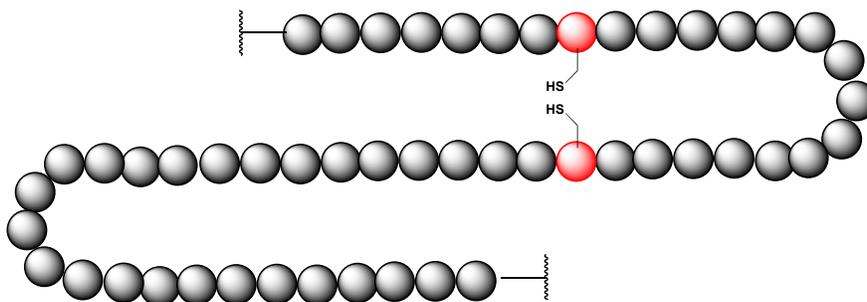
A **disulfide bond** is a sulfur-sulfur bond, usually formed from two free thiol groups.



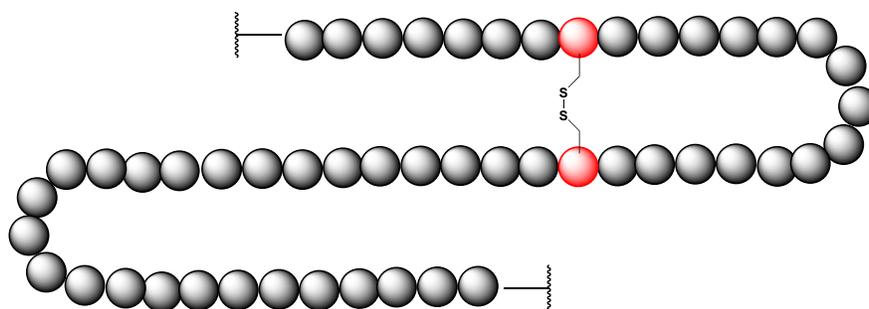
The interconversion between dithiol and disulfide groups is a redox reaction: the free dithiol form is in the reduced state, and the disulfide form is in the oxidized state. Notice

that in the oxidized (disulfide) state, each sulfur atom has lost a bond to hydrogen and gained a bond to sulfur.

As you should recall from your Biology courses, disulfide bonds between cysteine residues are an integral component of the three-dimensional structure of many extracellular proteins and signaling peptides.

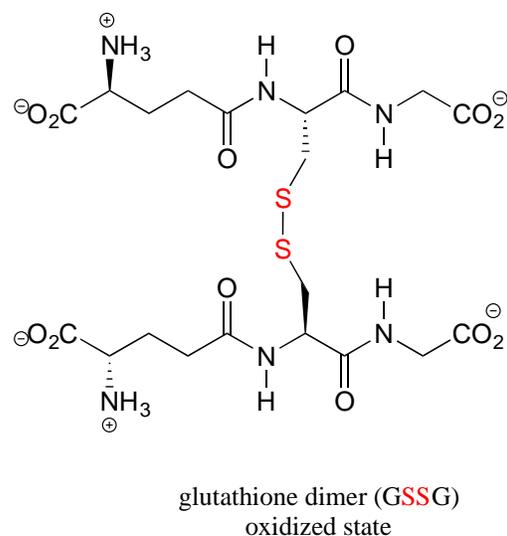
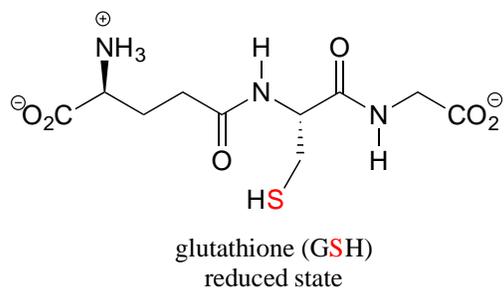


reduced protein (free cysteines)



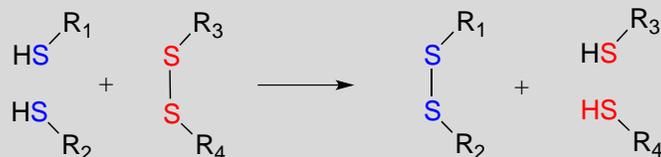
oxidized protein (disulfide)

A thiol-containing coenzyme called **glutathione** is integrally involved in many thiol-disulfide redox processes (recall that glutathione was a main player in this chapter's introductory story about concussion research). In its reduced (thiol) form, glutathione is abbreviated 'GSH'. In its oxidized form, glutathione exists as a dimer of two molecules linked by a disulfide group, and is abbreviated 'GSSG'.

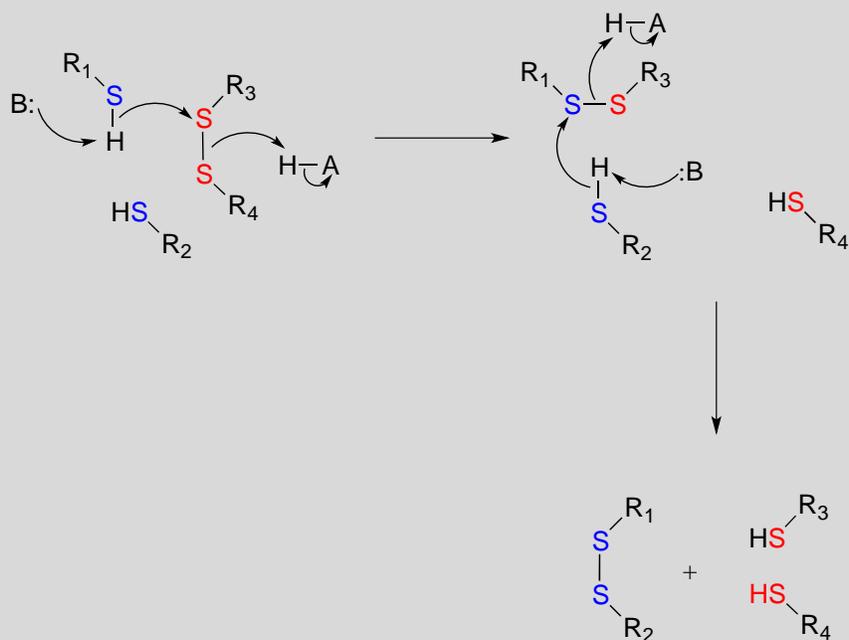


Disulfide bonds and free thiol groups in both proteins and smaller organic molecules like glutathione can 'trade places' through a **disulfide exchange** reaction. This process is essentially a combination of two direct displacement (S_N2 -like) events, with sulfur atoms acting as nucleophile, electrophile *and* leaving group.

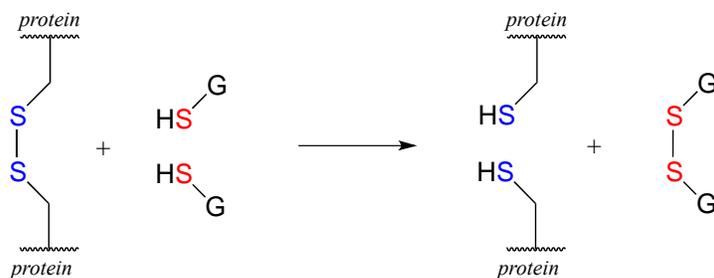
Disulfide exchange reaction



Mechanism:

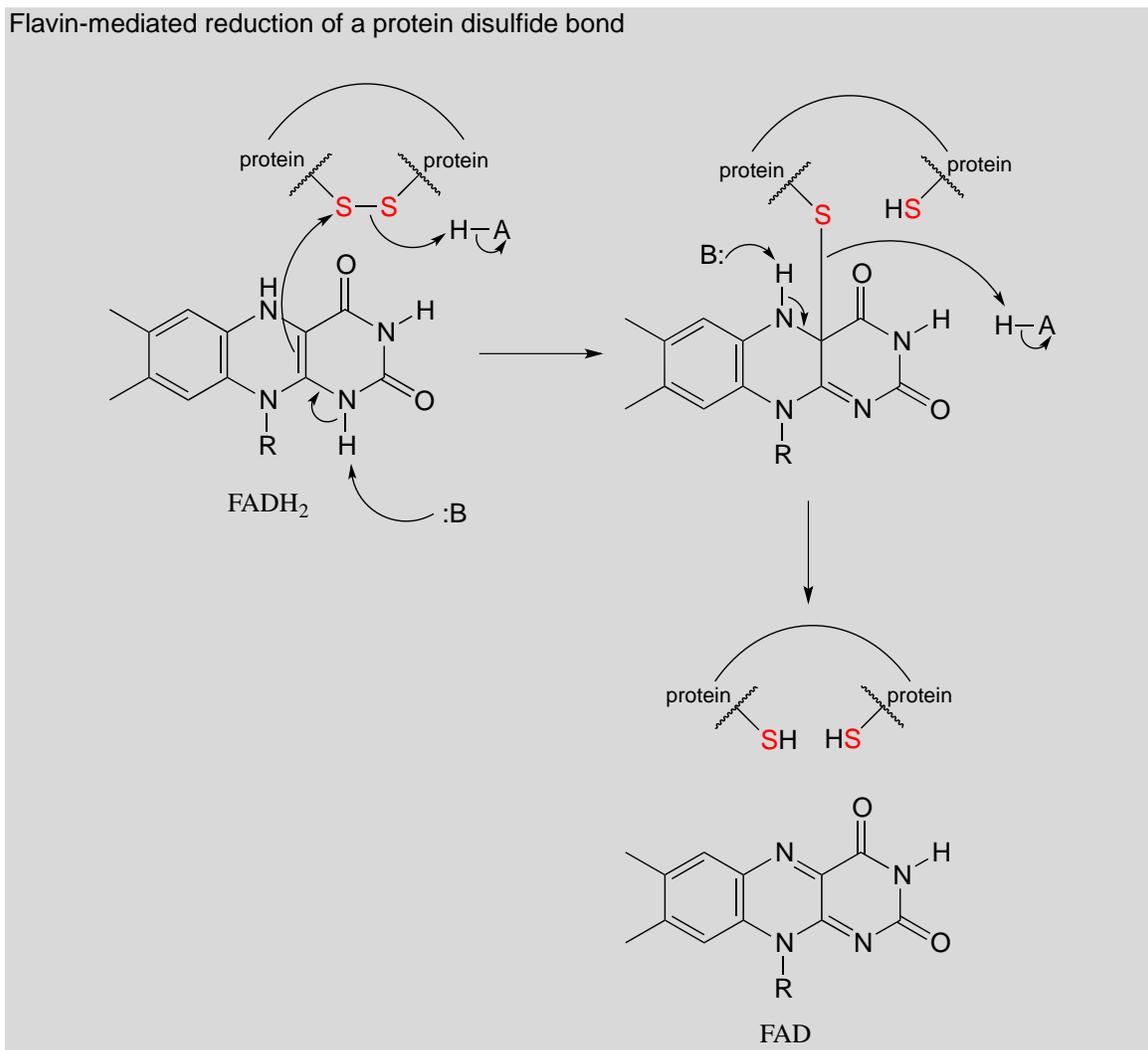


In eukaryotes, the cysteine side chains of intracellular (inside the cell) proteins are almost always in the free thiol (reduced) state due to the high concentration of reduced glutathione (GSH) in the intracellular environment. A disulfide bond in an intracellular protein will be rapidly reduced in a disulfide exchange reaction with excess glutathione.

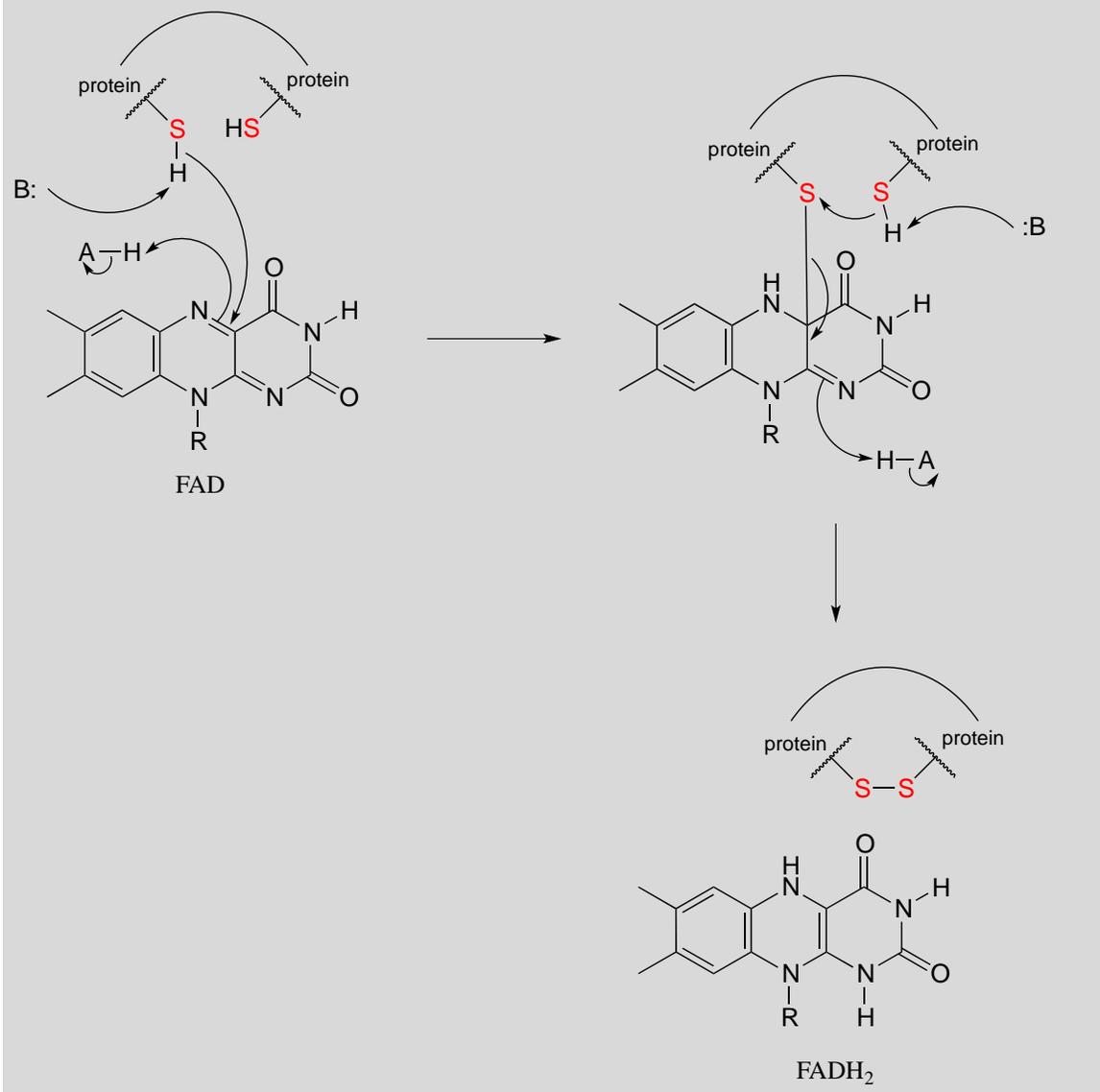


The interconversion of free thiols and disulfides is also mediated by flavin in some enzymes.

Flavin-mediated reduction of a protein disulfide bond

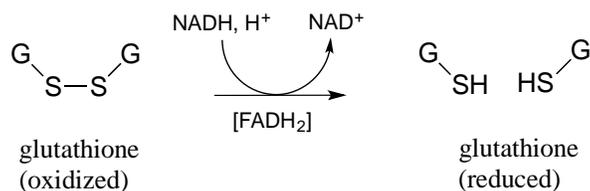


Flavin-mediated oxidation of a protein disulfide bond



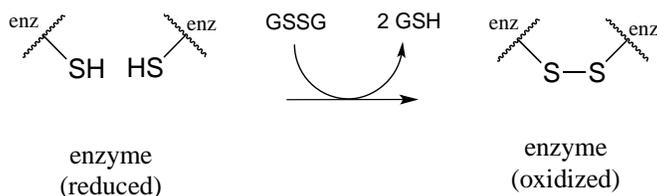
As was stated earlier, a high intracellular concentration of reduced glutathione (GSH) serves to maintain proteins in the free thiol (reduced) state. An enzyme called glutathione reductase catalyzes the reduction of GSSG in a flavin-mediated process, with NADH acting as the ultimate hydride donor.

Glutathione reductase reaction:

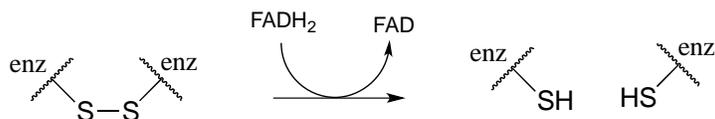


The mechanism for this and other similar reactions is not yet completely understood, but evidence points to an initial thiol-disulfide exchange reaction with a pair of cysteines from the enzyme, (phase 1 below) followed by flavin-dependent reduction of the cysteine-cysteine disulfide (phase 2). Finally, (phase 3) FAD is reduced back to FADH₂ by NADH.

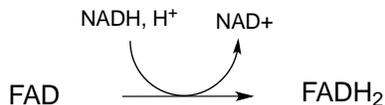
Phase 1: thiol-disulfide exchange (*see earlier figure for mechanism*):



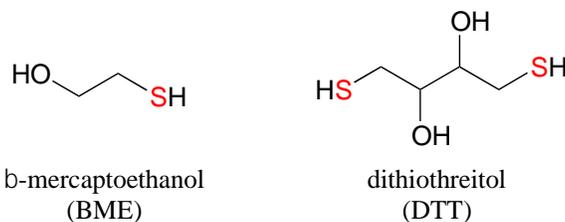
Phase 2: Reduction of protein disulfide by FADH₂ (*see earlier figure for mechanism*)



Phase 3: regeneration of FADH₂ by NADH (*see section 15.4B for mechanism*)



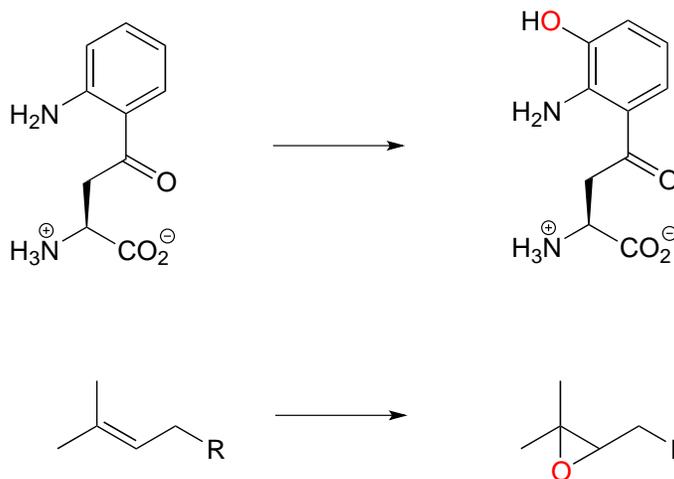
In the biochemistry lab, proteins are often maintained in their reduced (free thiol) state by incubation in buffer containing an excess concentration of β -mercaptoethanol (BME) or dithiothreitol (DTT). These reducing agents function in a manner similar to that of GSH, except that DTT, because it has two thiol groups, can form an *intramolecular* disulfide in its oxidized form.



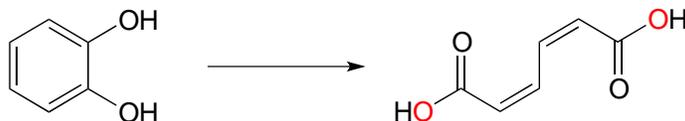
Exercise 15.14: Draw structures of the oxidized (disulfide) forms of BME and DTT.

Section 15.7: Flavin-dependent monooxygenase reactions: hydroxylation, epoxidation, and the Baeyer-Villiger oxidation

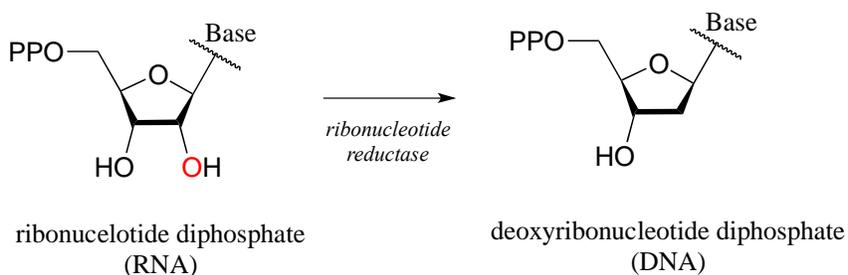
Up to now, the redox reaction examples we have seen have all been either hydrogenation/dehydrogenation transformations or interconversions between free thiols and disulfides. However, there are many important redox reactions in biological chemistry which do not fall under either of these descriptions. **Oxygenase** enzymes catalyze the insertion of one or two oxygen atoms from molecular oxygen (O_2) into an organic substrate molecule. Enzymes which insert a single oxygen atom are called **monooxygenases**. Below are two examples of biochemical transformations catalyzed by monooxygenase enzymes: one is a hydroxylation, the other is an epoxidation (an **epoxide** functional group is composed of a three-membered carbon-carbon-oxygen ring - epoxides are somewhat rare in biological organic chemistry but are very common and useful intermediates in laboratory organic synthesis).



Dioxygenase enzymes insert both oxygen atoms from O_2 into the substrate, and usually involve cleavage of an aromatic ring. Below is an example of a dioxygenase reaction, catalyzed by catechol dioxygenase:



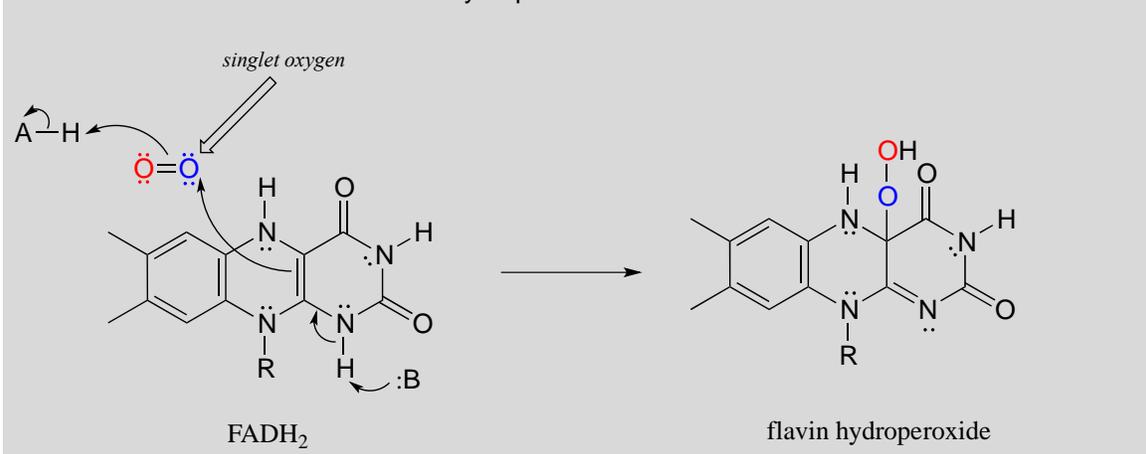
In the reduction direction, reductases *remove* oxygen atoms, or sometimes other electronegative heteratoms such as nitrogen or halides. For example, DNA **deoxy**ribonucleosides are converted from their corresponding RNA ribonucleosides by the action of reductase enzymes:



Many oxygenase and reductase reactions involve the participation of enzyme-bound transition metals - such as iron or copper - and the mechanistic details of these reactions are outside the scope of our discussion. A variety of biochemical monooxygenase reactions, however, involve flavin as a redox cofactor, and we do have sufficient background knowledge at this point to understand these mechanisms. In flavin-dependent monooxygenase reactions, the key intermediate species is **flavin hydroperoxide**.

The term 'peroxide' refers to a functional group characterized by an oxygen-oxygen single bond. The simplest peroxide is hydrogen peroxide (HOOH) about which we will have more to say below. In flavin hydroperoxide, the peroxide group is linked to one of the carbons of the reactive triple-ring system of the coenzyme. A possible mechanism for the formation of flavin peroxide from FADH₂ and molecular oxygen is shown below.

Mechanism for the formation of flavin hydroperoxide:



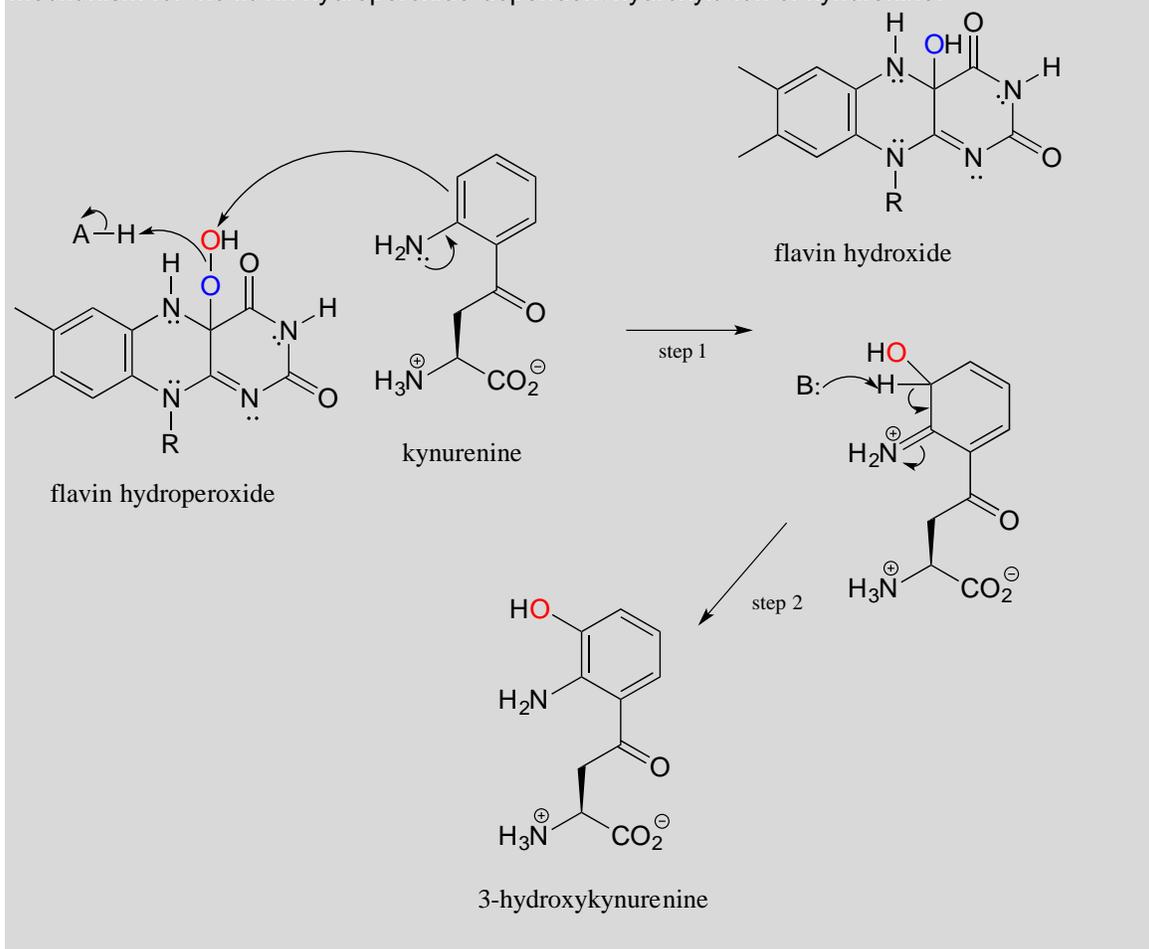
(Note: Implicit in this mechanism is that the molecular oxygen first undergoes spin inversion from the triplet state to the higher energy 'singlet' state. You may recall from your general chemistry course that molecular oxygen exists in two states: 'singlet' oxygen has a double bond and no unpaired electrons, while 'triplet' oxygen has a single O-O bond and two unpaired electrons - a kind of 'double radical'. Molecular orbital theory - and experimental evidence - show that the triplet state is lower in energy.

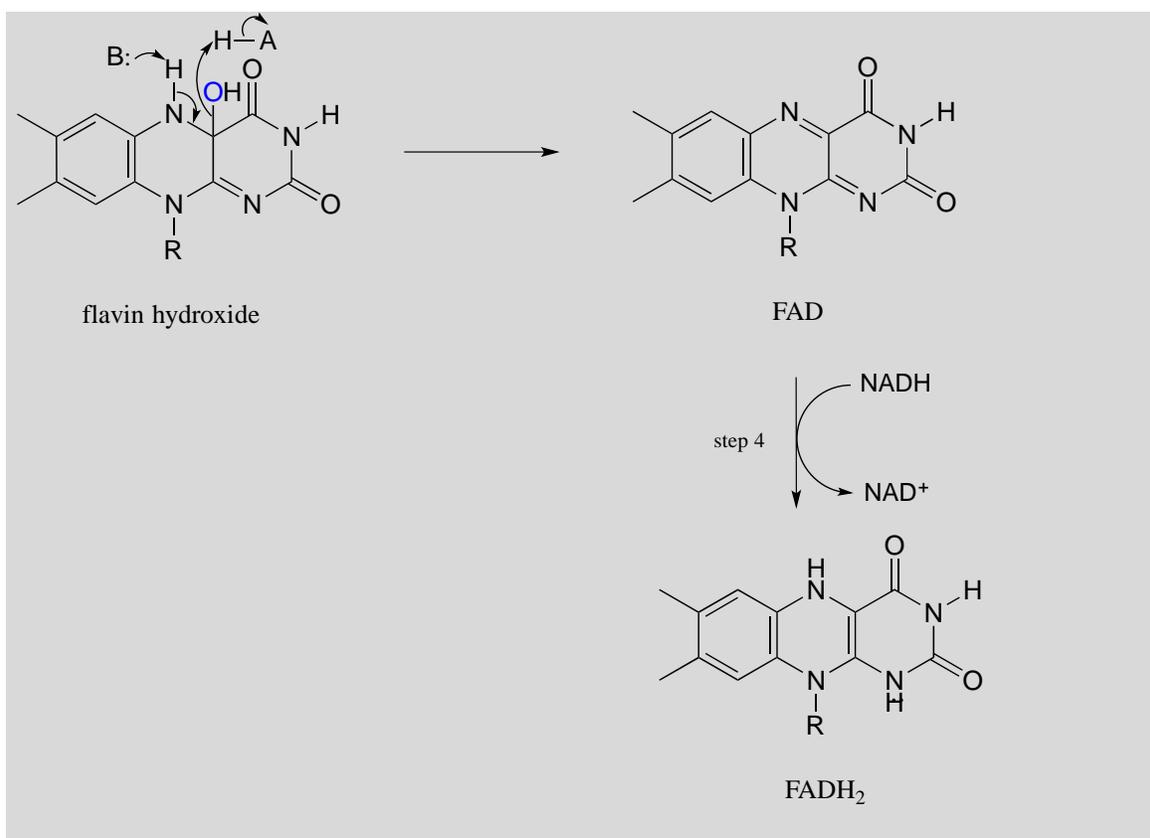


The mechanism shown above is one proposed mechanism, another proposal involves triplet oxygen reacting with flavin in a series of radical-intermediate, single-electron steps.)

Flavin hydroperoxide can be thought of as an activated form of molecular oxygen. ***Peroxides in general are potent oxidizing agents, because the oxygen-oxygen single bond is quite weak:*** only 138 kJ/mole, compared to 339 kJ/mol for a carbon-carbon bond, and 351 kJ/mol for a carbon-oxygen bond. When the 'outer' oxygen of flavin hydroperoxide (red in our figure above) comes into close proximity to the π -bonded electrons of an alkene or aromatic group, the O-O bond will break, leaving an empty orbital on the outer oxygen to be filled by the π electrons - thus, a new carbon-oxygen bond is formed. This is what is happening in step 1 of a reaction in the tryptophan degradation pathway catalyzed by kynurenine 3-monooxygenase. Step 2 completes what is, mechanistically speaking, an electrophilic aromatic substitution reaction (section 14.4) with an peroxide oxygen electrophile.

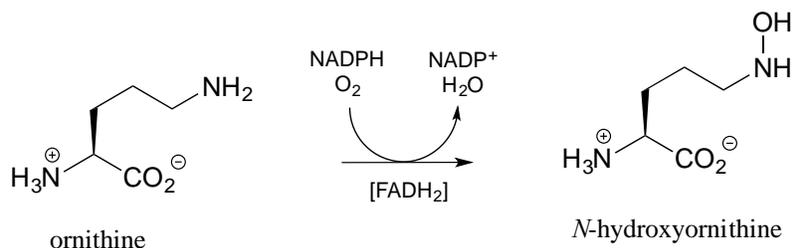
Mechanism for the flavin hydroperoxide-dependent hydroxylation of kynurenine:





Elimination of water from the hydroxyflavin intermediate then leads to formation of FAD (step 3), which is subsequently reduced back to FADH₂ by NADH (step 4).

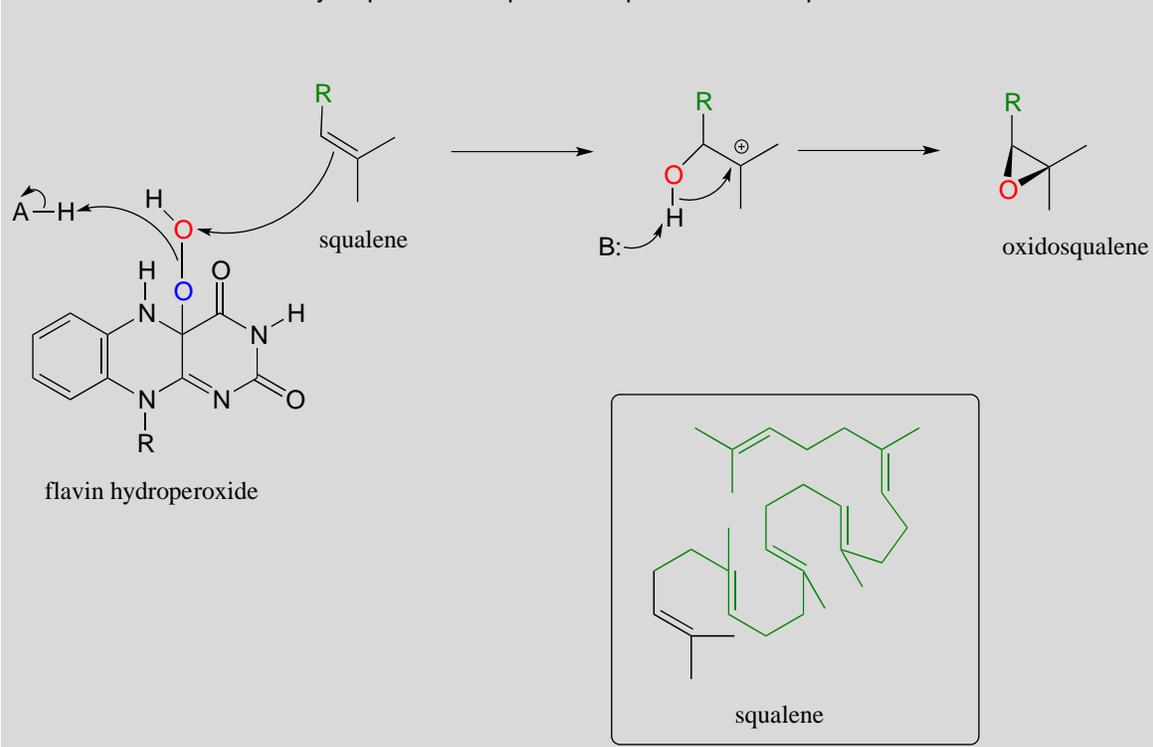
The *N*-hydroxylation reaction below, which is part of the biosynthetic pathway of an iron-binding molecule in the pathogenic bacterium *Pseudomonas aeruginosa*, is mechanistically similar to the *C*-hydroxylation reaction we just saw, except that the nucleophile is an amine nitrogen. Note that FADH₂ is shown in brackets below the reaction arrow, indicating that reduced flavin participates in the reaction but is not used up - rather it is regenerated in the active site at the end of the reaction cycle.



Exercise 15.15: Draw arrows for the N-O bond-forming step in the ornithine hydroxylation reaction above.

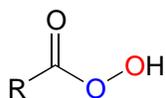
Epoxides, characterized by a three-membered ring composed of two carbons and one oxygen, are a very common and useful functional group employed in synthetic organic chemistry. Although rare, there are some interesting epoxide-forming reactions in biochemical pathways, catalyzed by flavin-dependent monooxygenase enzymes. In a key step in the biosynthesis of cholesterol and other steroid compounds, an alkene is converted to an epoxide in a precursor molecule called squalene. Flavin hydroperoxide also serves as the direct oxidizing agent in this step:

Mechanism for the flavin-hydroperoxide-dependent epoxidation of squalene:

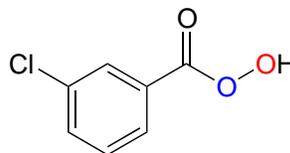


Oxidosqualene goes on to cyclize to lanosterol in a complex and fascinating electrophilic reaction which we discussed in section 14.5.

Epoxidation reactions have a parallel in the synthetic organic laboratory, and in fact are very important tools in organic synthesis. In laboratory epoxidations, **peroxyacids** are the counterpart to flavin hydroperoxide in biochemical epoxidations. *meta*-chloroperoxybenzoic acid (MCPBA) is a commonly used peroxyacid.

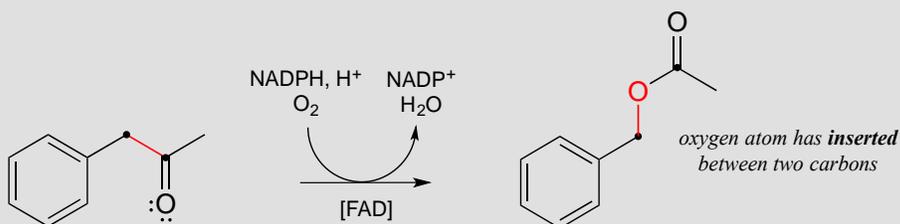


a peroxyacid

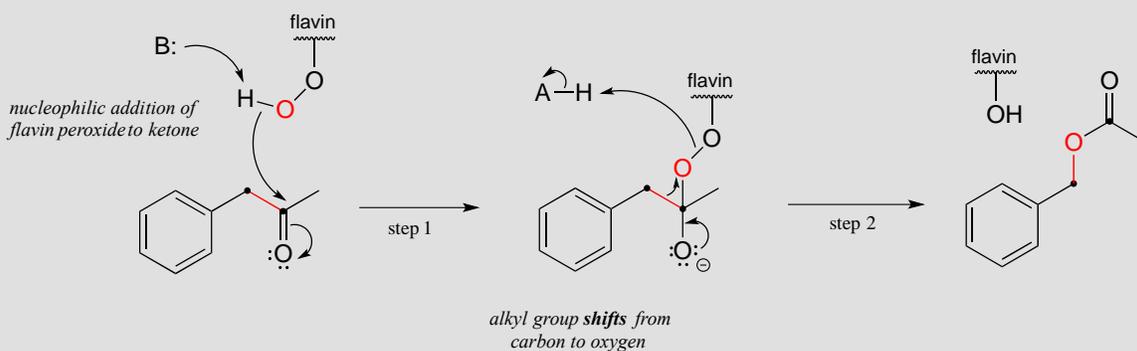
*meta*-chloroperoxybenzoic acid
(MCPBA)

The Baeyer-Villiger oxidation, in which a ketone is converted to an ester through treatment with a peroxide reagent, is an extremely useful laboratory organic synthesis reaction discovered in the late 19th century. Recently, many biochemical examples of Baeyer-Villiger oxidations have been discovered: the reaction below, for example, is catalyzed by a monooxygenase in a thermophilic bacterium: (*Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13157)

A Baeyer-Villiger oxidation:



Mechanism:



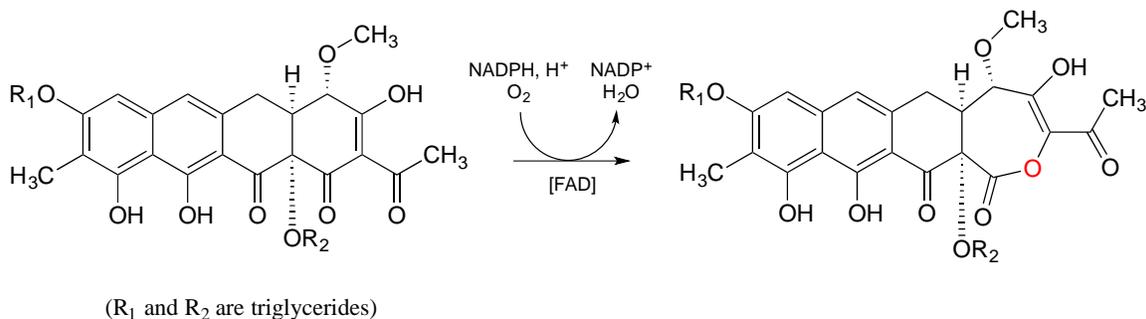
The Baeyer-Villiger mechanism differs significantly from the hydroxylation reactions we saw earlier, although flavin hydroperoxide (abbreviated in the above figure) still plays a key role. Here, the peroxide oxygen is a nucleophile, rather than an electrophile, attacking the ketone carbonyl in step 1. Step 2 is a rearrangement, similar in many ways to the hydride and alkyl shifts we learned about in section 14.5. The electrons in the red bond in the figure shift over one atom: from the carbonyl carbon to the outer peroxide oxygen. The end result is that an oxygen atom, from O_2 via flavin hydroperoxide, has been inserted between the carbonyl carbon and a neighboring methylene (CH_2) carbon, forming an ester.

Note that in the reaction mechanism above, the ketone substrate is *asymmetric*: on one side of the carbonyl there is a benzyl group (CH₂-phenyl), and on the other side a methyl group. Note also that it is the benzyl group, not the methyl, that shifts in step 2 of the mechanism. For reasons that are not yet well understood, in Baeyer-Villiger reactions the alkyl group with higher carbocation stability has a higher **migratory aptitude**: in other words, it has a lower energy barrier for the shifting step.

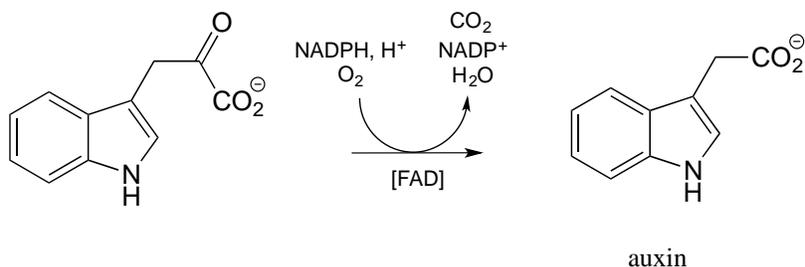
Exercise 15.16: Draw the product of a *hypothetical* Baeyer-Villiger reaction involving the same substrate as the above figure, in which the methyl rather than the benzyl group shifts.

Exercise 15.17: Draw the likely major product of a hypothetical Baeyer-Villiger reaction starting with 2-methylcyclopentanone as the substrate. Take into account the idea of migratory aptitude.

Below is another example of a Baeyer-Villiger reaction in which a cyclic ketone is oxidized to a lactone (cyclic ester). Notice that oxygen insertion expands the ring from 6 to 7 atoms. This is the third-to-last step in the biosynthesis of the anti-cancer agent mithromycin in some bacterial species (*ACS Chem. Biol.* **2013**, *8*, 2466).



Yet another variety of flavin-dependent monooxygenase, which bears some mechanistic similarity to the Baeyer-Villiger oxidation, is the decarboxylative reaction below from biosynthesis of the plant hormone auxin: (*J. Biol. Chem.* **2013**, *288*, 1448)



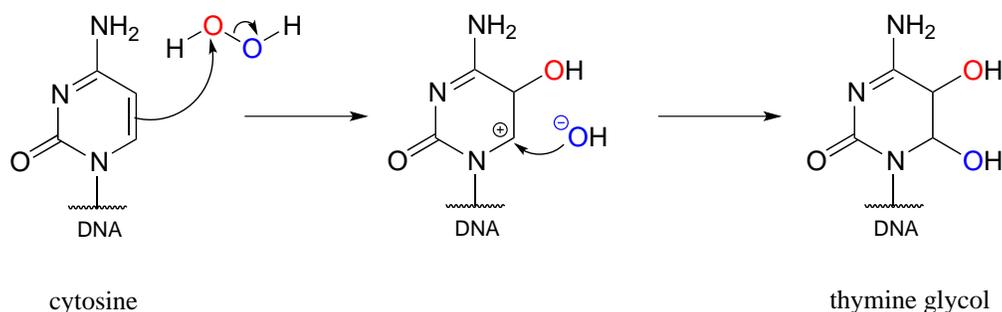
Exercise 15.18: Propose a mechanism for the above reaction, starting with flavin hydroperoxide.

Section 15.8: Hydrogen peroxide as a dangerous Reactive Oxygen Species

We get our energy from the oxidation of organic molecules such as fat and carbohydrates, as electrons from these reduced compounds are transferred to molecular oxygen, thereby reducing it to water. Reducing O_2 , however, turns out to be a hazardous activity: harmful side products called **reactive oxygen species (ROS)** are inevitably formed in the process. Recall from the story introducing this chapter that ROS appear to play an important role in the damage that occurs to the brain immediately after a concussion.

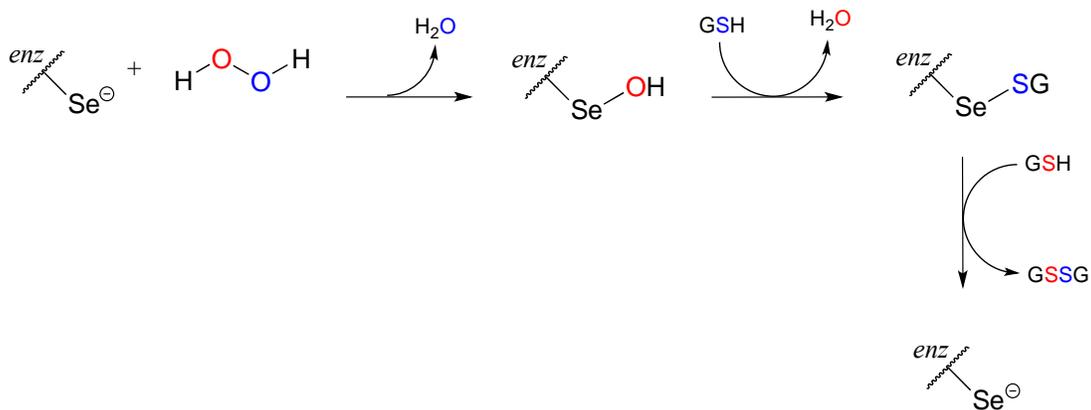
Hydrogen peroxide, HO_2H , is an ROS. Recall peroxides are potent oxidizing agents due to the weakness of the O-O single bond. It is this same weak bond that causes hydrogen peroxide to be dangerous when produced in our bodies, as it can react spontaneously with oxygen or nitrogen nucleophiles and π bonds.

Peroxide formed as a by-product of our metabolism is particularly harmful when it oxidizes DNA bases. In just one of many known examples of oxidative damage, the DNA base cytosine is oxidized to thymine glycol in the presence of hydrogen peroxide. Although mechanistic details for reactions such as these are not yet well understood, one possibility is electrophilic addition:



Our bodies have evolved ways to dispose of the harmful reactive oxygen species that are continuously being formed (the only way to stop the production of ROS is to stop breathing oxygen!). Glutathione peroxidase is a remarkable enzyme in that its active site contains selenocysteine, a modified cysteine residue in which the side chain sulfur is replaced by selenium (selenium is very toxic, but we do need a very small amount of it in our diet). Look at a periodic table: selenium is below oxygen and sulfur in the same column. If you think back to the vertical periodic trends in nucleophilicity (section 8.2B), you'll recall that just as a thiol is a better nucleophile than an alcohol, a selenol ($RSeH$) is even more nucleophilic than a thiol. Moreover, the vertical periodic trend in acidity (section 7.3A) tells us that a selenol should be more acidic than a thiol - in fact, the pK_a of a selenocysteine is about 5.5, meaning that it is mostly in its deprotonated state at physiological pH, making it even more nucleophilic.

Glutathione peroxidase very efficiently catalyzes the reduction of hydrogen peroxide to water and the oxidation of glutathione (GSH) to GSSG, beginning with nucleophilic attack by the enzymatic selenocysteine on a peroxide oxygen. The intermediates in this process are shown below: each step can be thought of as a concerted nucleophilic displacement similar to those that take place in a disulfide exchange reaction.



Reactive oxygen species in our cells can also be beneficial in certain contexts. Immune cells called macrophages and neutrophils engulf pathogenic bacteria, then generate high intracellular concentrations of ROS to help destroy them.

Key learning objectives for this chapter

Before moving on to the next chapter, you should be able to:

Recognize when an organic molecule is being oxidized or reduced, and distinguish between redox and non-redox organic reactions.

Draw complete mechanisms for the following reaction types, including the structure of the reactive part of the redox coenzyme (*it is strongly recommended that you commit to memory the structures of the reactive parts of the nicotinamide and flavin coenzymes*).

- oxidation of an alcohol to an aldehyde or ketone
- oxidation of an amine to an imine
- oxidation of an aldehyde to a carboxylic acid derivative (usually a thioester or carboxylate)
- oxidation of an alkane to an alkene at the α,β position relative to a carbonyl or imine
- reduction of an aldehyde or ketone to an alcohol
- reduction of an imine to an amine
- reduction of a carboxylic acid derivative to an aldehyde
- reduction of an α,β - conjugated alkene to an alkane
- oxidation of two thiol groups to a disulfide in a disulfide-exchange type reaction
- reduction of a disulfide group by flavin
- flavin hydroperoxide-dependent hydroxylation, epoxidation, and Baeyer-Villiger reactions
- reduction of FAD (or FMN) to FADH₂ (or FMNH₂) by NAD(P)H.
- spontaneous oxidation of an alkene group in a biomolecule by hydrogen peroxide
- reduction of hydrogen peroxide by glutathione peroxidase

In addition, you should be able to draw complete mechanisms for hydrogenation-dehydrogenation and disulfide exchange reactions that we have not yet seen specific examples of, based on your understanding of the chemistry involved in these reaction types and organic reaction patterns in general. Several exercises and end-of-chapter problems provide opportunities practice with inferring and drawing mechanisms of less familiar redox reactions.

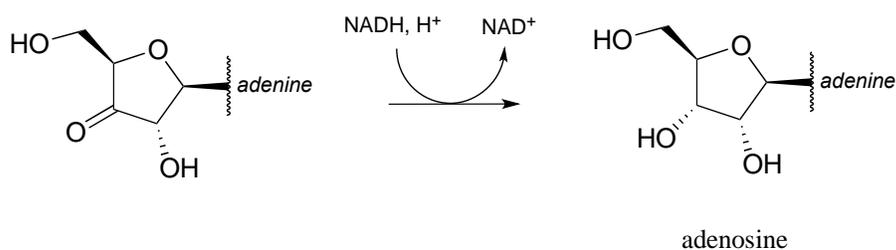
Given a multistep pathway diagram, you should be able to recognize the transformations taking place and fill in missing intermediate compounds or reagents (problem 15.5 is an example of this type).

You should be working on gaining proficiency at solving multi-step pathway elucidation problems, such as those at the end of this chapter's problem section.

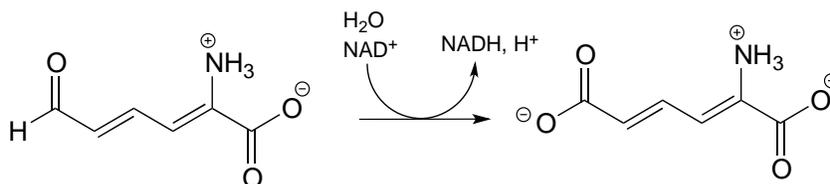
Problems

P15.1: Show a mechanism for each of the redox reactions below. Do not abbreviate the reactive parts of the redox coenzyme.

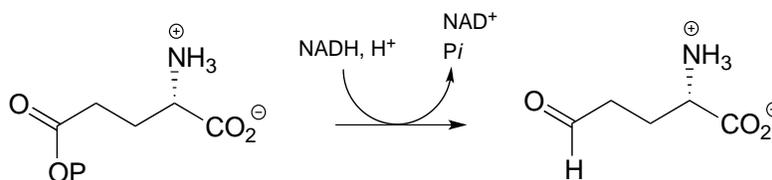
a)



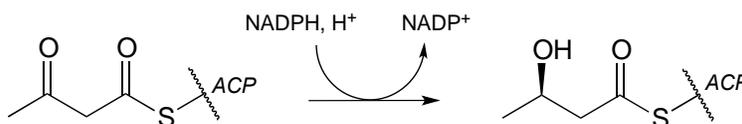
b)



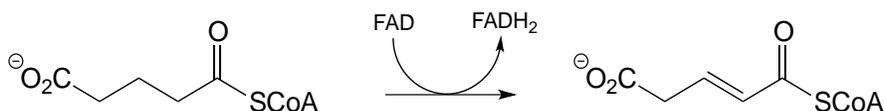
c)



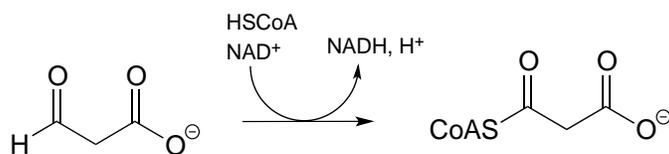
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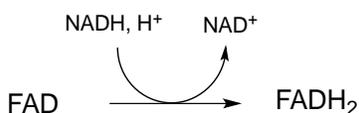
e)



f)



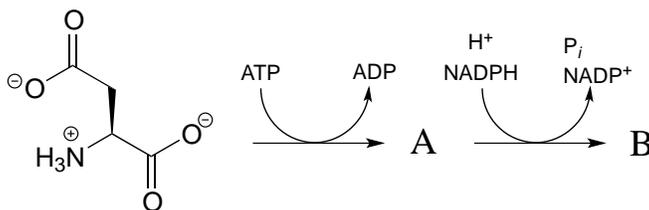
g) (regeneration of reduced flavin by NADH):



h) In reaction (a), near which face of the substrate is the cofactor bound in the active site?

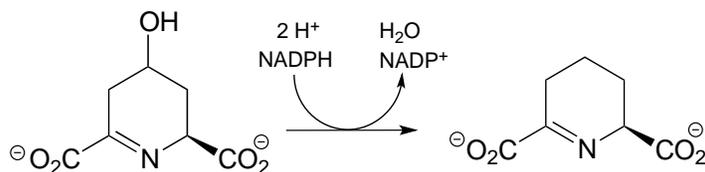
i) In reaction (d), near which face of the product is the cofactor bound in the active site?

P15.2: In the reactions below ((EC 2.7.2.4; EC 1.2.1.11), the side chain of aspartate is altered, but the main peptide chain is not affected. Show the most probable structure of species A and B.

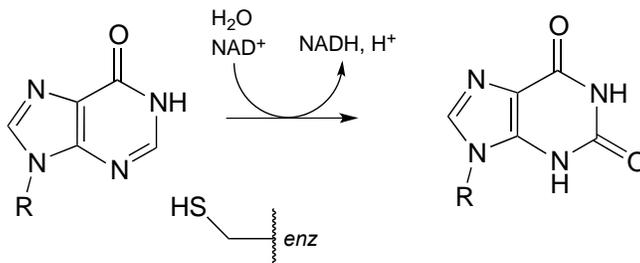


P15.3: Propose complete mechanisms for the following reactions.

a) (EC 1.3.1.26, from lysine biosynthesis)

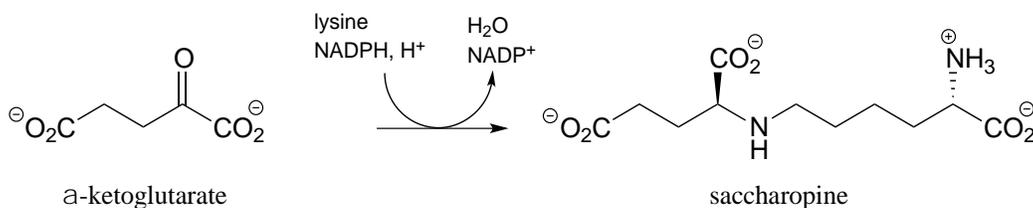


b) (EC 1.1.1.205, from guanosine ribonucleotide biosynthesis) The mechanism involves a covalent cysteine-linked enzyme-substrate intermediate.

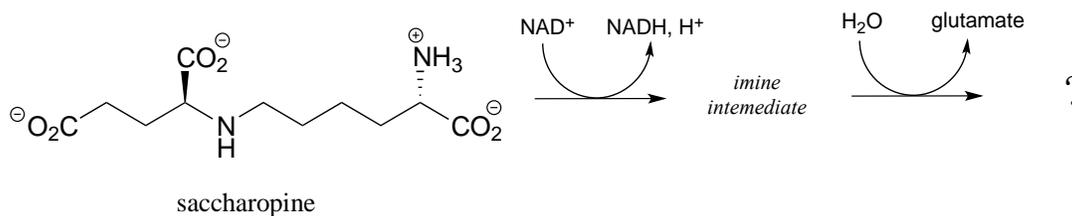


P15.4: The first step in the lysine degradation pathway is a reductive condensation with α -ketoglutarate to form an intermediate called saccharopine. (EC 1.5.1.8)

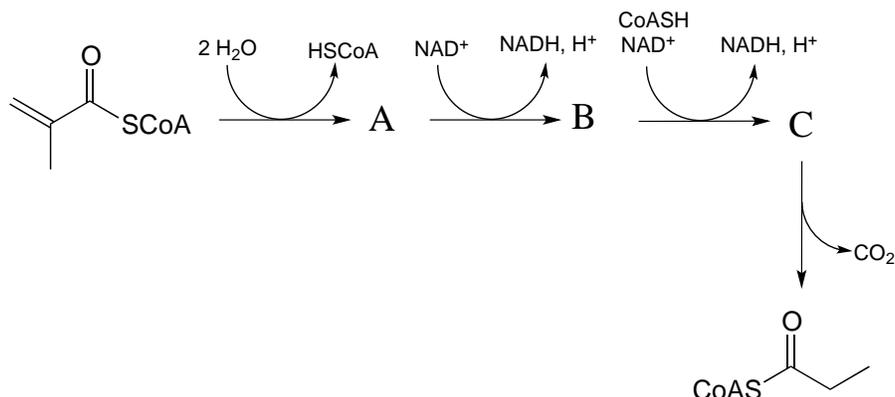
a) Propose a mechanism for this transformation.



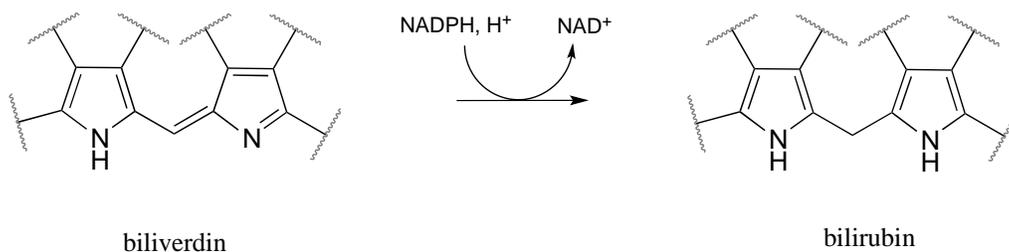
b) Saccharopine (see part (a) above) is then broken up to yield glutamate and a second product that contains an aldehyde group. Predict the structure of this second product, and propose a likely mechanism for the reaction which involves an imine intermediate. (EC 1.5.1.10)



P15.5: Predict the structures of species A and B in the pathway below.

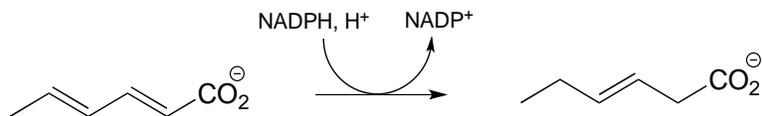


P15.6: Bilirubin, the molecule responsible for the yellowish color of bruises, is formed from the NADPH-dependent hydrogenation of a double bond in biliverdin (EC 1.3.1.24), which is a product of heme degradation (heme is an iron-containing coenzyme in the oxygen-carrying blood protein hemoglobin). Draw a likely mechanism for this reaction.

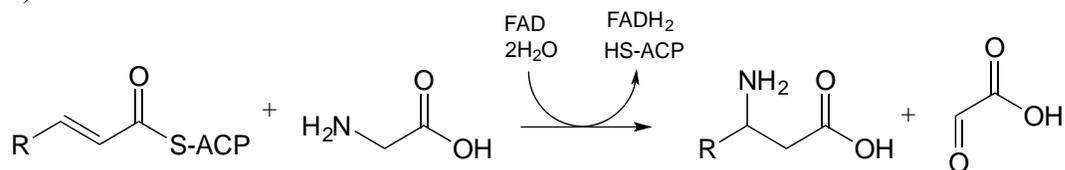


P15.7: Draw a likely mechanism for each of the reactions below.

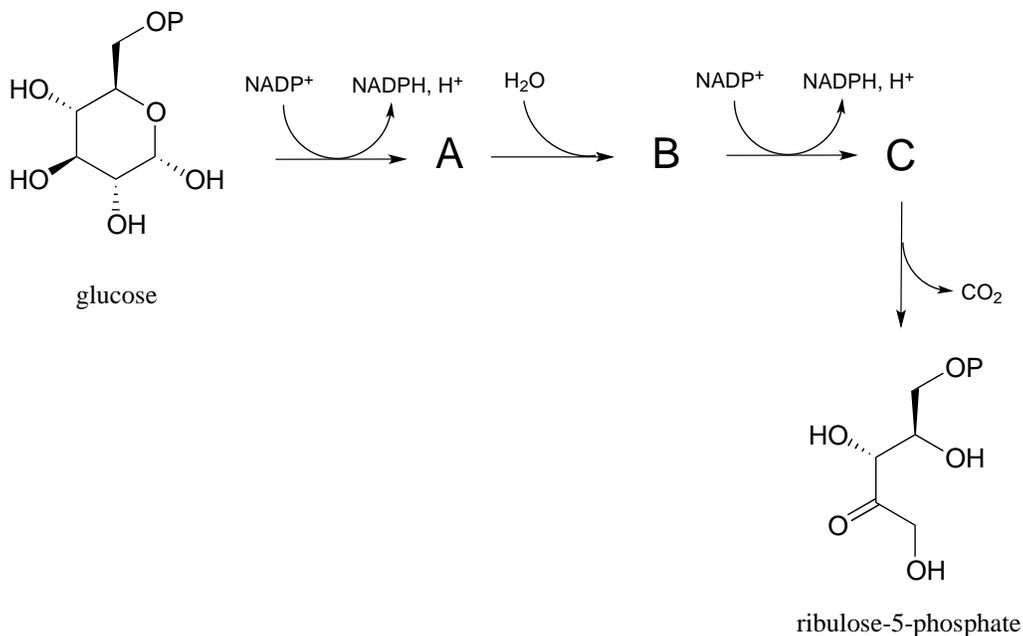
a) From the oxidation of polyunsaturated fatty acids:



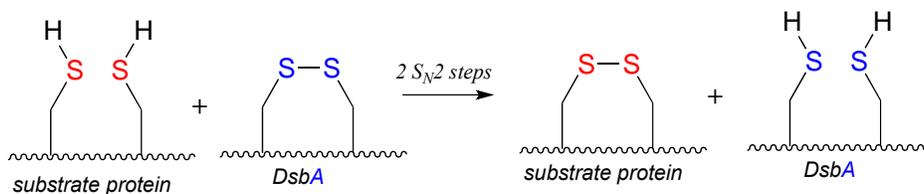
b)



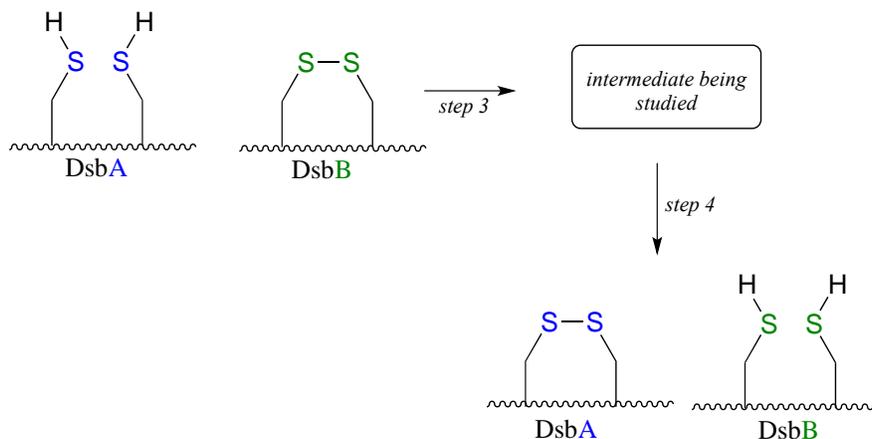
P15.8: Predict the structures of pathway intermediates A, B, and C:



P15.9: An enzyme called DsbA (EC1.8.4.2) is responsible for the formation of disulfide bonds in bacterial proteins. The process - which can be thought of as a 'disulfide exchange', involves the cleavage of a disulfide bond between two active site cysteines in DsbA. It is accomplished through two successive S_N2 displacements.



DsbA is then returned to its starting (disulfide) state through a second disulfide exchange reaction with another protein called DsbB:



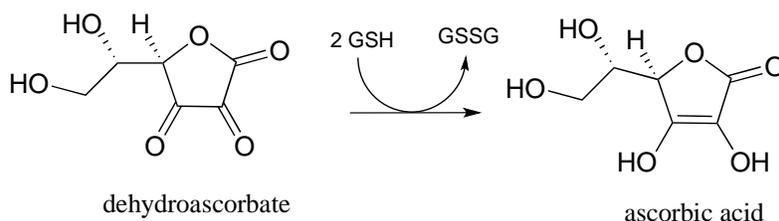
Scientists were interested in studying the intermediate species formed in step 3, but found that it is very short-lived and difficult to isolate. In order to address this problem, they ran the reaction with a synthetic analog of DsbB that contained an unnatural bromoalanine amino acid in place of one of the active site cysteines.

a) Draw a complete mechanism for the disulfide exchange reaction between DsbA and DsbB.

b) Show how the bromoalanine-containing DsbB analog allowed for the isolation of an intermediate that resembles the true, short-lived intermediate.

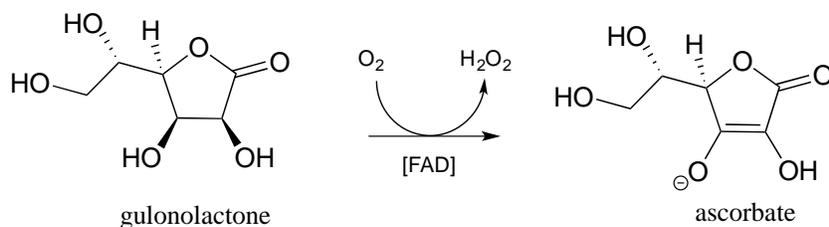
P15.10:

a) In chapter 16 we will learn how ascorbate (vitamin C) acts as a 'radical scavenger' antioxidant to protect our cells from damage by free radical species. When ascorbate scavenges a radical, it ends up being converted to dehydroascorbate. One possible metabolic fate of dehydroascorbate is to be recycled back to ascorbate through an enzyme-free reaction with glutathione.



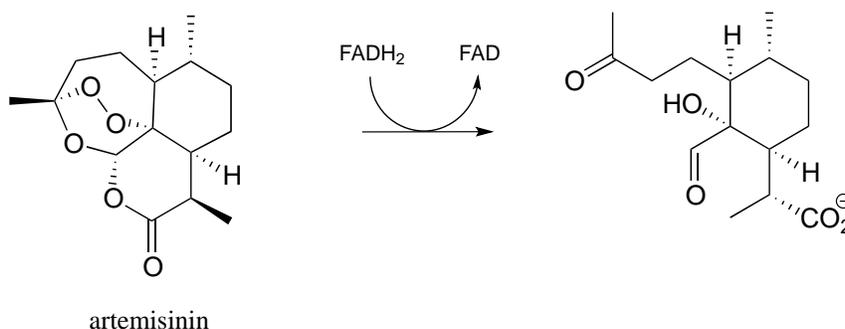
Suggest a likely mechanism for the enzyme-free reaction.

b) In the introduction to chapter 16, we will learn that most animals - but not humans - are able to synthesize their own ascorbate. Humans cannot synthesize vitamin C because we lack the enzyme for the final step in the biosynthetic pathway, gulonolactone oxidase:



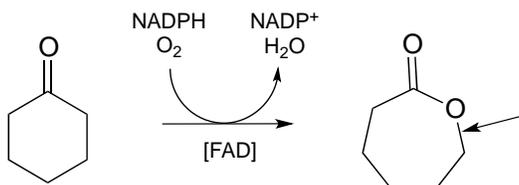
This enzyme uses FAD as an oxidizing agent, and FADH₂ is oxidized back to FAD at the end of the catalytic cycle by molecular oxygen, with hydrogen peroxide as a side product. Draw out a likely mechanism showing how gulonolactone is converted to ascorbate, and how FAD is regenerated.

c) Artemisinin is a naturally occurring compound with demonstrated antimalarial properties. It is thought to act by depleting the malaria-causing microbe's store of reduced flavin, thus disrupting the redox balance. The relevant reaction is shown below:

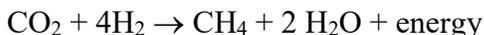


Draw mechanistic arrows for the step as shown above.

d) Draw the mechanistic step in the reaction below in which the C-O bond indicated by the arrow in the figure below is formed.



P15.11: Methanogens are a class of microorganisms in the domain archaea which inhabit a diversity of anaerobic (oxygen-lacking) environments, from the intestines of humans, to swamp mud, to the base of deep sea hot water vents. They obtain energy by reducing carbon dioxide to methane:



Methanogenesis', like the oxidation of glucose in animals, is not accomplished in a single reaction - it requires a long series of enzymatic steps, and involves the participation of several unique coenzymes (if you are interested in learning more, see *FEMS Microbiol. Rev.* **1999**, *23*, 13 for a detailed review of the enzymatic reactions of methanogenesis).

The oxidation of methane to carbon dioxide when you burn natural gas for heating your house is obviously an exergonic process. How then is it possible that reducing carbon dioxide to methane could also be exergonic? Explain.

Multistep pathway prediction problems

In chapters 12 and 13, we were introduced to the challenge of mapping out potential multi-step transformations using the retrosynthesis approach, where we start with the more complex molecule and take it apart step by step. These problems have necessarily involved hypothetical, generalized transformations, because many of the reaction types involved in actual biochemical transformations were as-yet unfamiliar to us.

We have now reached a point in our study of organic reactivity where we can look at an actual metabolic pathway and probably recognize *most* of the reactions taking place - so we are ready to try our hand at mapping out real metabolic pathways.

Before we dive in, you may want to go back to the retrosynthesis interchapter and review the key elements of the retrosynthetic approach. You may (or may not) also need a little more practice with some simpler, shorter, hypothetical problems, similar to what we worked on in the last three chapters but incorporating some of the redox reactions that we have just finished learning about. These are provided in problems 15.12 and 15.13 below.

As before, your job is to draw out a 'pathway diagram' for each transformation, using the 'arrow in - arrow out' convention to indicate the role of other necessary participants in each reaction, such as ATP, NADH, water, or another organic molecule. An example for a simple two-step pathway is provided in problem 15.12 below. A three-step pathway would of course show *two* intermediate compounds.

Remember, it is most important that your proposed pathway be *chemically reasonable* - in other words, each hypothetical reaction that you propose should be very similar to a reaction pattern that we have seen in chapters 8-15. You should be able to put a name on each step: for example, 'step 1 is a Claisen condensation; step 2 is a ketone reduction', and so forth.

Also remember that there is usually no one correct way to approach problems like this - they are puzzles to solve, and success will be dependent in large part on having a solid grasp on the chemical 'tools' available to us: in other words, the biochemical reaction types that we have been studying, starting with nucleophilic substitutions in chapter 8.

P15.12: hypothetical 2-step transformations:

Each of the generalized transformations below would be expected to require *two* enzymatic steps. Draw a reasonable pathway diagram for each transformation.

Example:

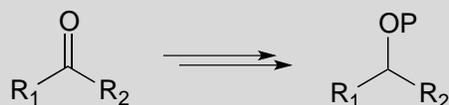
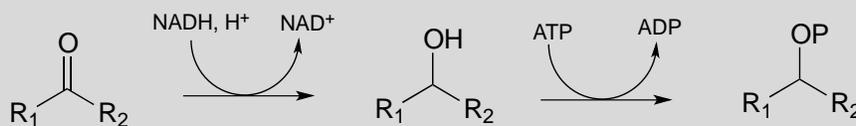
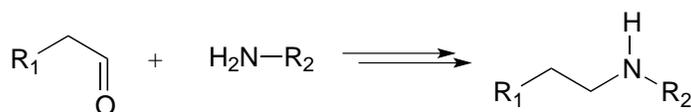


Diagram:

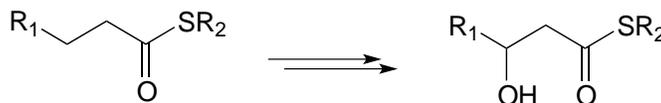


The first step is a nicotinamide-dependent ketone reduction/hydrogenation, and step 2 is ATP-dependent phosphorylation (ie. a kinase reaction). Note that the reducing agent in the first step could also be NADPH

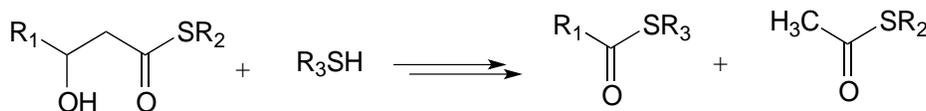
a)



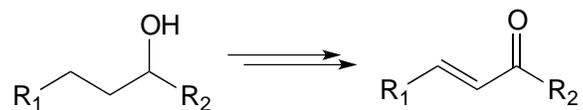
b)



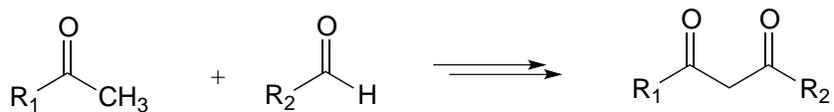
c)



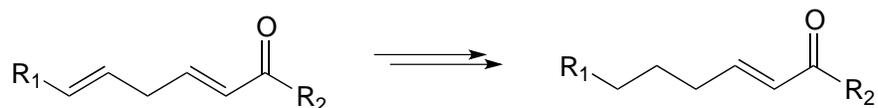
d)



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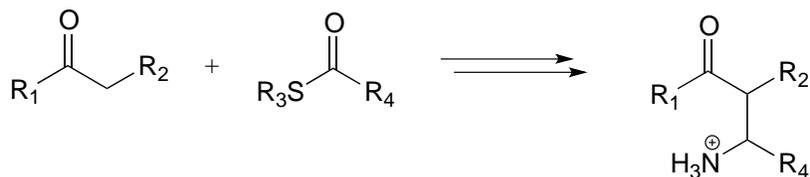


f)

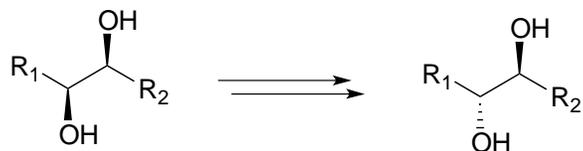
**P15.13:** hypothetical 3-step transformations:

Each of the generalized transformations below would be expected to require three enzymatic steps. Draw a reasonable pathway diagram for each transformation.

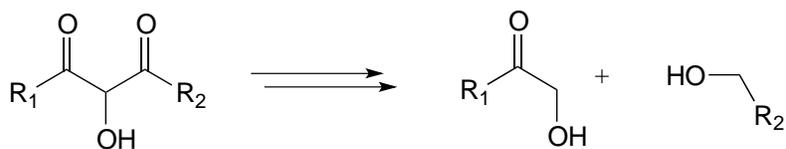
a)



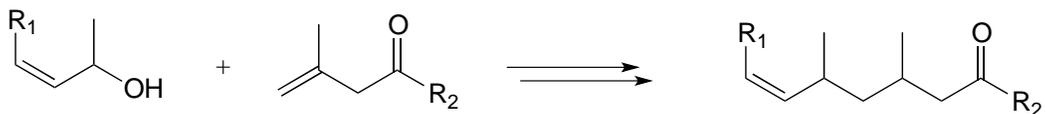
b)



c)



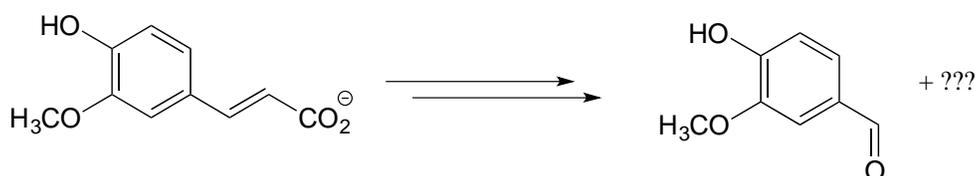
d)



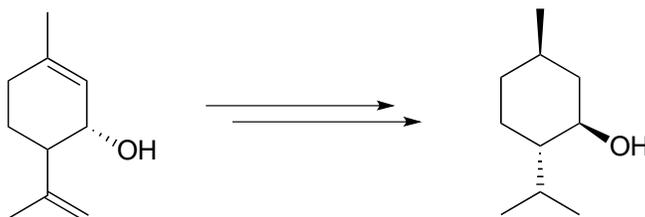
P15.14: Now, let's try our hand at predicting the steps of some actual metabolic pathways. For each, draw a complete pathway diagram. As needed, use any of the coenzymes we have studied, water, and ammonia.

Note: you will probably find these quite challenging! Do not expect to be able to figure them out in a few minutes - rather, think of them as puzzles to work on over a period of time, sharing ideas and strategies with classmates. Remember, if one side of the transformation is larger or more complex, start there and work towards the simpler molecules. It is also a good idea, when applicable, to start the process by a) counting carbons on each side of the transformation, and b) identifying the key bond being formed (or broken) in the transformation.

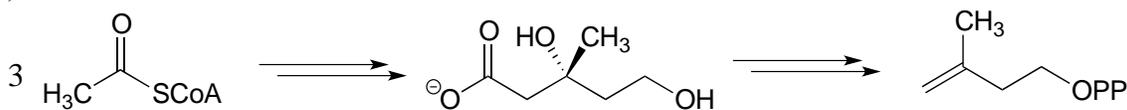
a)



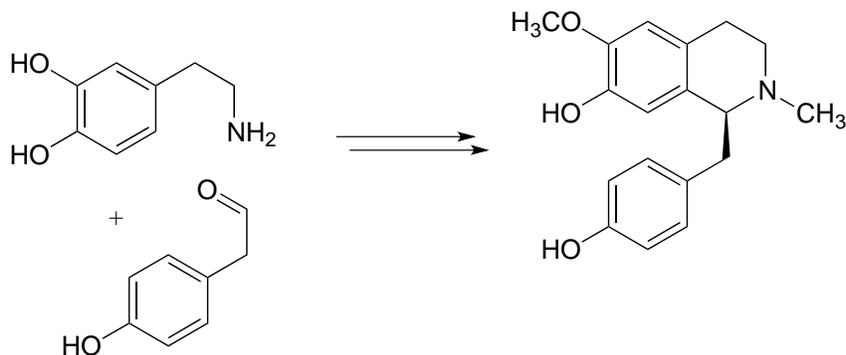
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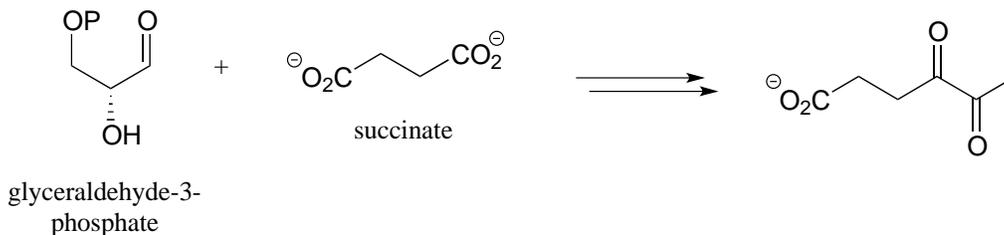
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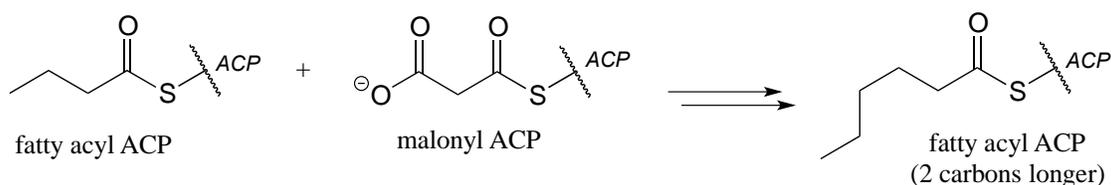


f)

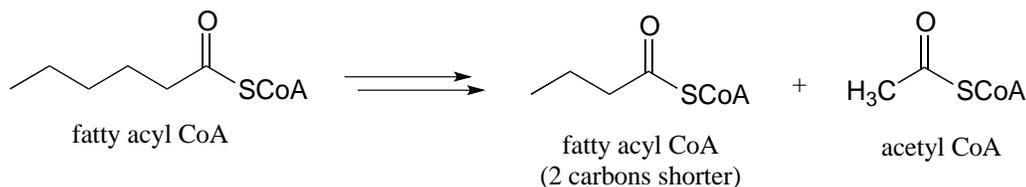


P15.15: Propose a pathway diagram for each of the metabolic pathways below. Note that some pathways contain steps that will be unfamiliar to you, and are therefore provided already.

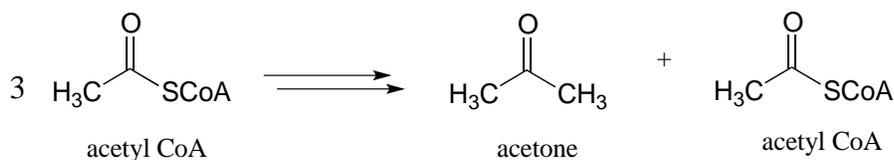
a) one cycle of fatty acid biosynthesis:



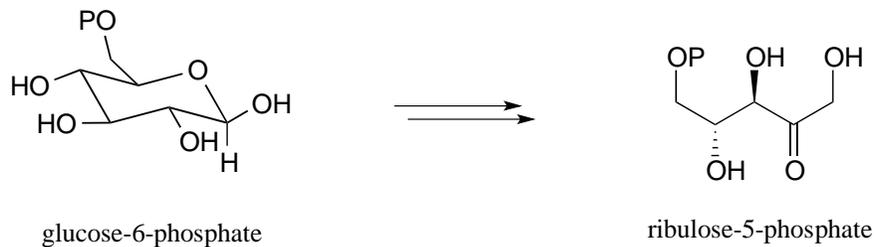
b) one cycle of fatty acid degradation:



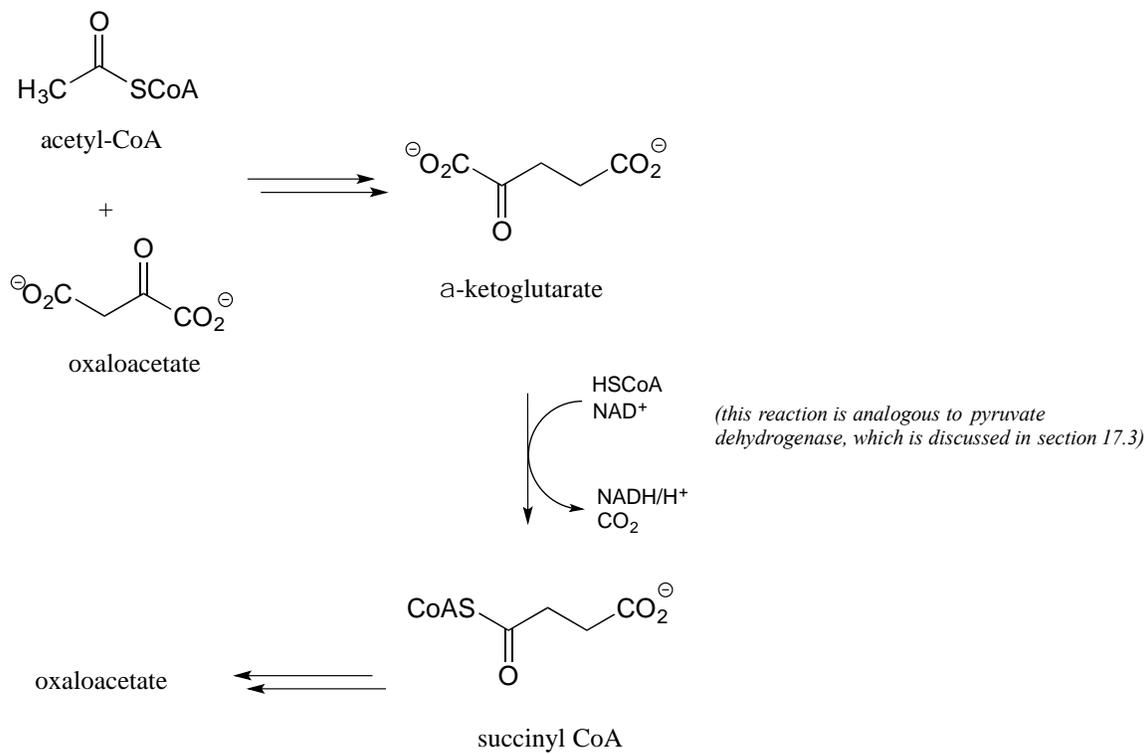
c) Diabetics and people who adopt an extreme low-carbohydrate diet sometimes have breath that smells like acetone, due to 'ketone body' formation that occurs when acetyl-CoA from fatty acid oxidation (see part (b) above) is not able to enter into the citric acid cycle. Draw a pathway diagram showing how three molecules of excess acetyl CoA combine to form acetone (all three acetyl-CoA molecules first link together, but one is left over at the end of the process).



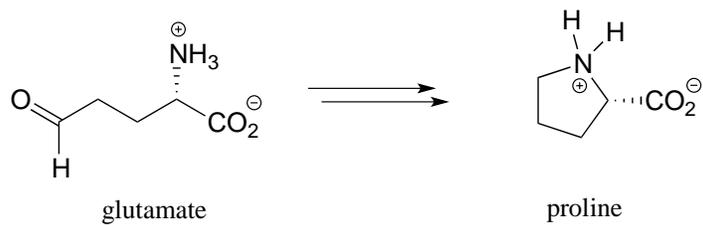
d) Pentose phosphate pathway (oxidative branch):



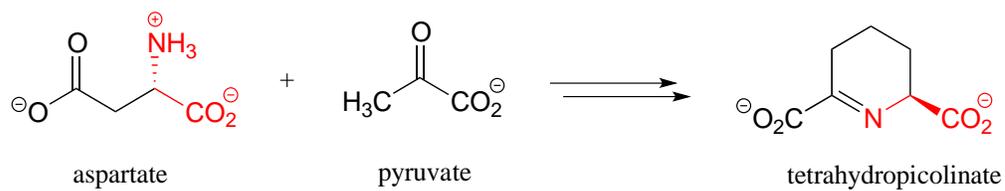
e) Citric acid (Krebs) cycle:



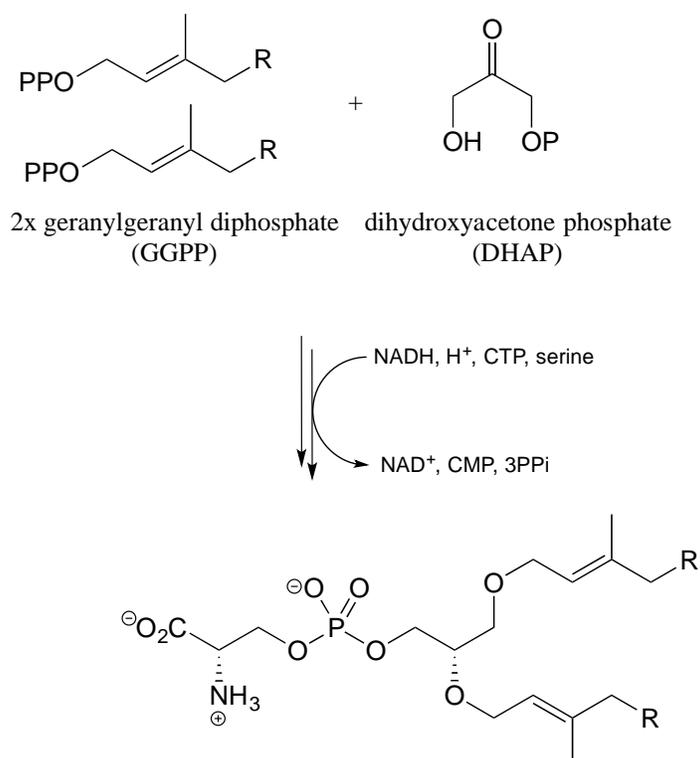
f) proline biosynthesis:



g) First half of lysine biosynthesis:



h) From the biosynthesis of membrane lipid in archaea



Chapter 16

Free radical reactions



photo credit: <https://www.flickr.com/photos/stevehopson/>

Introduction

Imagine that you are an 18th century British sailor about set out with Commodore George Anson to raid Spanish shipping fleets in the Pacific. You know full well that you are signing up for a long and arduous ordeal, with months of constant seasickness, bad food, cramped, unsanitary conditions, and brutal warfare. You are mentally ready for these

hardships, but what you are not prepared for is to watch your own body rot away – to literally fall apart.

Below is a description of the suffering endured by many sailors of the time:

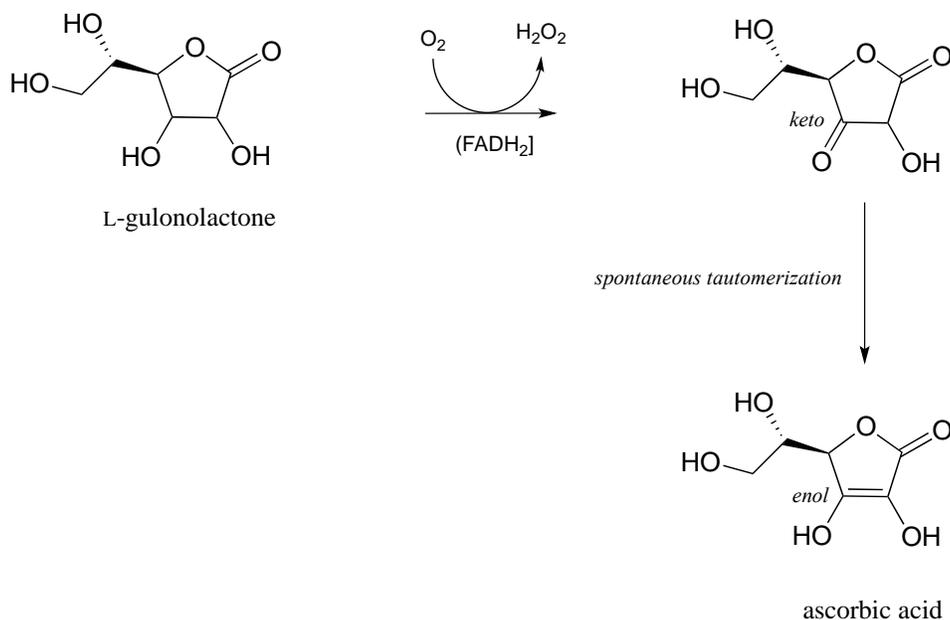
Some lost their very substance and their legs became swollen and puffed up while the sinews contracted and turned coal-black and, in some cases, all blotched with drops of purplish blood. Then the disease would creep up to the hips, thighs and shoulders, arms and neck. And all the sick had their mouths so tainted and their gums so decayed that the flesh peeled off down to the roots of their teeth, which almost all fell out. . .

There were devastating neurological as well as physiological effects. Scurvy had the ability to inhibit a person's normal restraints on emotion: they became intensely homesick and nostalgic, wept at the slightest disappointment, and screamed in agony upon smelling the scent of flower blossoms drifting across the water from a nearby shore.

The disease afflicting the sailors was scurvy, which we now know is caused by a deficiency of vitamin C in the diet. European sea voyagers in the 18th century and earlier subsisted mainly on a diet of salted meat, hard biscuits, pea soup, oatmeal, and beer. After the first couple of weeks at sea, fresh fruits and vegetables - and the nutrients they contained – were all consumed or spoiled. The salted meat and hardtack diet provided salt and calories, but little else of nutritional value.

Although it is rare now, scurvy has plagued sailors for centuries, with records of its occurrence on ships going back as far as the 15th century voyages of Magellan and Vasco de Gama, both of whom lost up to three quarters of their crew to the disease on long ocean crossings. Various cultures made the connection between scurvy and diet, and learned effective preventative measures: sailors with the 16th century French explorer Jacques Cartier, for example, were cured of their scurvy upon arriving in Canada and taking the advice of native people to eat the leaves and bark of pine trees. These were lessons, unfortunately, that often had to be relearned time and again, as the knowledge gained by one culture was not effectively recorded and passed along to others.

Vitamin C, or ascorbic acid as it is known to chemists, plays an essential helping role in a variety of essential biochemical reactions. Most living things are able to synthesize ascorbic acid – the exceptions include humans and other higher primates, several species of bats, and some rodents such as guinea pigs and capybaras. Humans lack the last enzyme in the ascorbic acid biosynthetic pathway, L-gulonolactone oxidase. (EC 1.1.3.8) (You were invited to propose the mechanism for this redox reaction in problem 15.10).



Because we cannot make our own ascorbic acid, we need to get it in our diet. It is abundant in many plant-based foods, citrus fruits in particular. The traditional diet of the Inuit people of the arctic region contains virtually no plant products, but vitamin C is obtained from foods such as kelp, caribou livers, and whale skin. For a time in the 18th century, the observation that citrus fruits quickly cured scurvy led to the practice of including in a ship's stores a paste prepared from boiled lemon juice. Unfortunately, ascorbic acid did not survive the boiling process, rendering the paste ineffective against scurvy. Captain James Cook, the legendary explorer and the first European to make it to the east coast of Australia and the Hawaiian islands, brought along sourkraut (fermented cabbage), a somewhat more effective vitamin C supplement. According to his own account, Cook's sailors at first refused to eat the pungent preparation, so the captain engaged in a little psychological trickery: he declared that it would only be served to officers. The enlisted sailors quickly took offense, and demanded their own sourkraut ration. Later, the British navy famously adopted the practice of adding lemon or lime juice to their ships' rum rations, leading to the birth of the slang term 'Limey' used by European and American sailors to refer to their British counterparts.

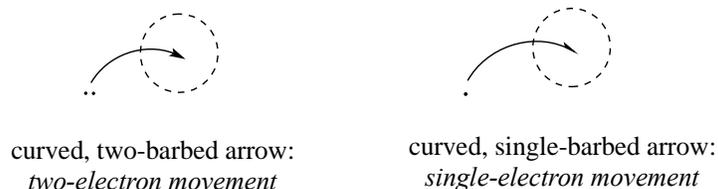
The biochemical role of ascorbic acid is to facilitate the transfer of *single electrons* in a variety of redox reactions - note here the emphasis on *single* electrons, as opposed to the redox reactions we studied in chapter 15 in which electrons were transferred in *pairs*. The subject of this chapter is single-electron chemistry, and the free radical intermediates that are involved in single electron reaction steps.

Later in this chapter we will learn the chemical details of why ascorbic acid deficiency causes scurvy, how the act of breathing makes you get old, how polystyrene packing foam is made, and other interesting applications of single-electron chemistry. But first

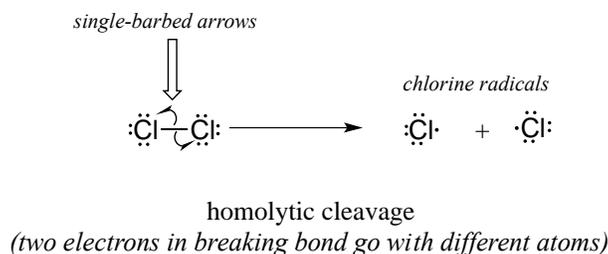
we need to cover some basic ideas about single electron chemical steps, and the free radical intermediates that result from them.

Section 16.1: Overview of single-electron reactions and free radicals

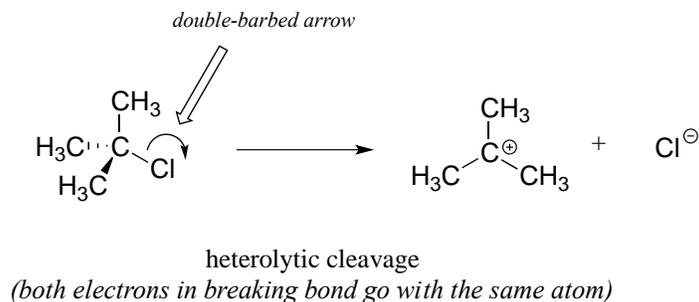
Beginning with acid-base reactions in chapter x and continuing through the chapters on nucleophilic substitution, carbonyl addition, acyl substitution, α -carbon chemistry, and electrophilic reactions, we have been studying reaction mechanisms in which *both* electrons in a covalent bond or lone pair move in the *same* direction. In this chapter, we learn about reactions in which the key steps involve the movement of *single* electrons. Single electron movement is depicted by single-barbed 'fish-hook' arrows (as opposed to the familiar double-barbed arrows that we have been using throughout the book to show two-electron movement).



Single-electron mechanisms involve the formation and subsequent reaction of **free radical** species, highly unstable intermediates that contain an unpaired electron. Free radicals are often formed from **homolytic cleavage**, an event in which the two electrons in a breaking covalent bond move in *opposite* directions. The bond in molecular chlorine, for example, is subject to homolytic cleavage when chlorine is subjected to heat or light. The result is two chlorine radicals. Note that each radical has a formal charge of zero.

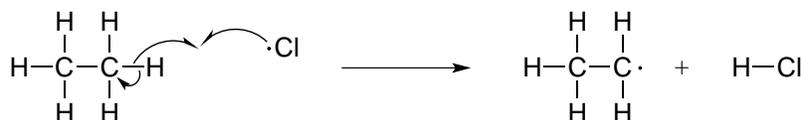


In contrast, essentially all of the reactions we have studied up to now involve bond-breaking events in which both electrons move in the same direction: this is called **heterolytic cleavage**.

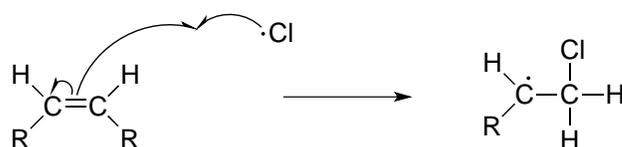


Two other homolytic cleavage reactions that we will see in this chapter can be described as 'radical hydrogen atom abstraction' and 'radical alkene addition':

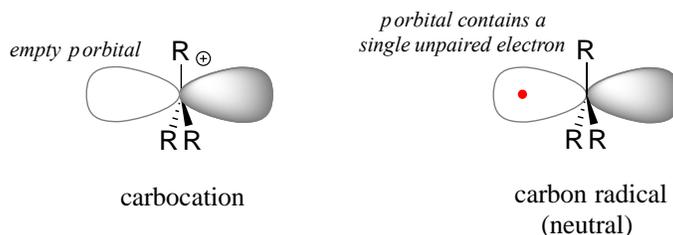
hydrogen abstraction by a radical



alkene addition by a radical

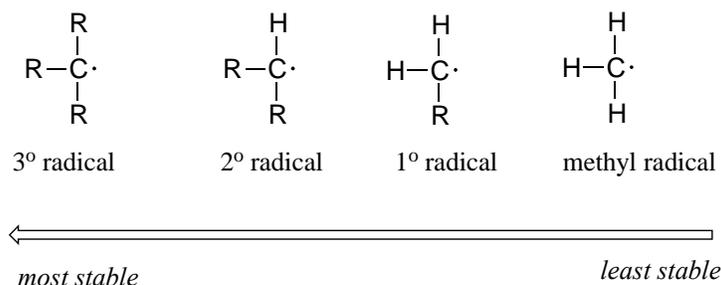


Single-electron reaction mechanisms involve the formation of radical species, and in organic reactions these are often carbon radicals. A carbon radical is sp^2 hybridized, with three σ bonds arranged in trigonal planar geometry and the single unpaired electron occupying an unhybridized p orbital. Contrast this picture with a carbocation reactive intermediate, which is also sp^2 hybridized with trigonal planar geometry but with an *empty* p orbital.

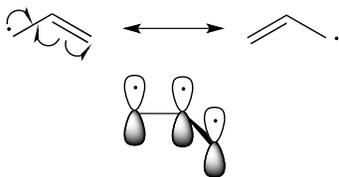


When we studied electrophilic reactions in chapter 14, a major concern when evaluating possible mechanisms was the stability of any carbocation intermediate(s). Likewise, the stability of proposed radical intermediates is of great importance when evaluating the

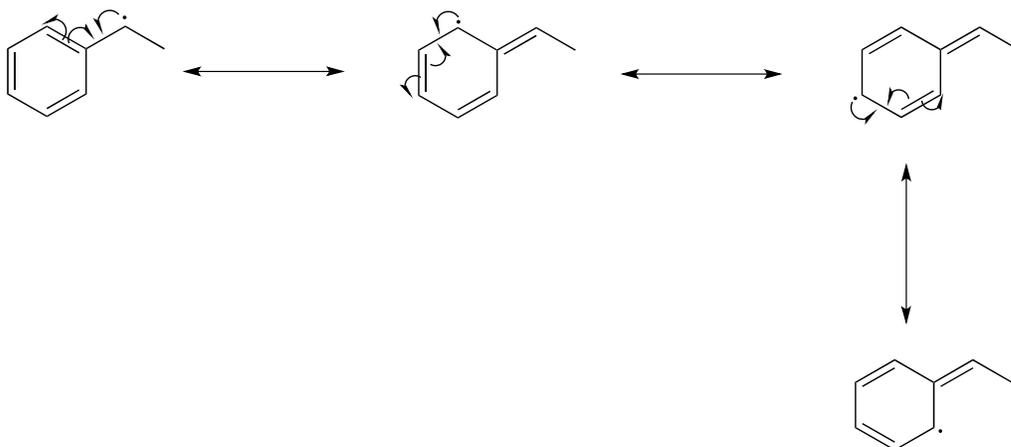
likelihood of possible single-electron mechanisms. Fortunately, the trend in the stability of carbon radicals parallels that of carbocations (section 8.5): tertiary radicals, for example, are more stable than secondary radicals, followed by primary and methyl radicals. This should make intuitive sense, because radicals, like carbocations, are electron deficient, and thus are stabilized by the electron-donating effects of nearby alkyl groups.



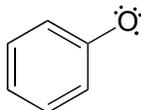
Benzylic and allylic radicals are more stable than alkyl radicals due to resonance effects - an unpaired electron (just like a positive or negative charge) can be delocalized over a system of conjugated π bonds. An allylic radical, for example, can be pictured as a system of three parallel p orbitals sharing three electrons.



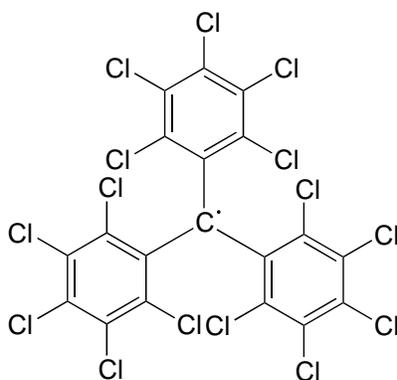
The drawing below shows how a benzylic radical is delocalized to three additional carbons around the aromatic ring:



Exercise 16.1: Just as phenolate ions are less reactive (less basic) than alkoxide ions, phenolic radicals are less reactive than alkoxide radicals. Draw one resonance contributor of a phenolic radical showing how the radical electron is delocalized to a ring carbon. Include electron-movement arrows.



While radical species are almost always very reactive and short-lived, in some extreme cases they can be unreactive. One example of an inert organic radical structure is shown below.



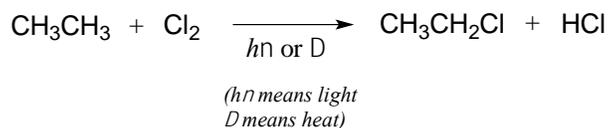
The already extensive benzylic resonance stabilization is further enhanced by the fact that the large electron clouds on the chlorine atoms shield the radical center from external reagents. The radical is, in some sense, inside a protective 'cage'.

Exercise 16.2: Draw a resonance contributor of the structure above in which the unpaired electron is formally located on a chlorine atom (include electron movement arrows)

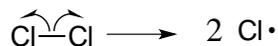
Section 16.2: Radical chain reactions

Because of their high reactivity, free radicals have the potential to be extremely powerful chemical tools - but as we will see in this chapter, they can also be extremely harmful in a biological/environmental context. Key to understanding many types of radical reactions is the idea of a **radical chain reaction**.

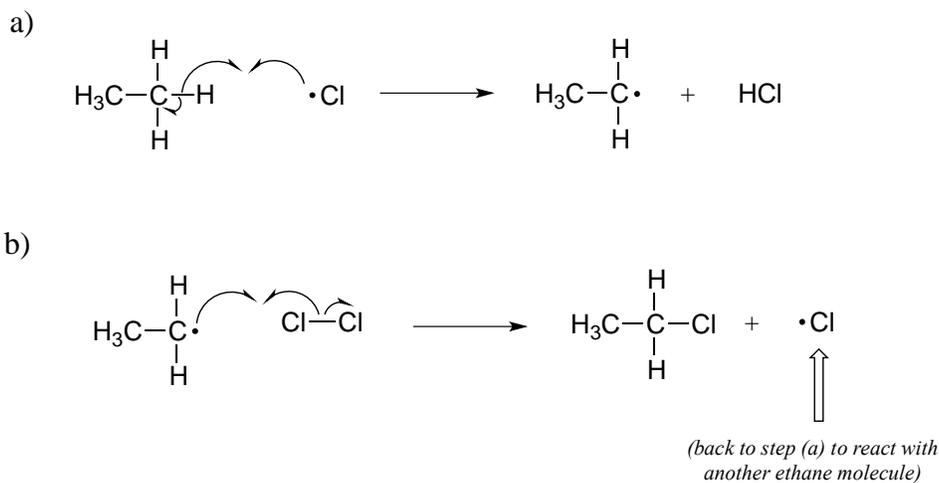
Radical chain reactions have three distinct phases: initiation, propagation, and termination. We'll use a well-known example, the halogenation of an alkane such as ethane, to illustrate. The overall reaction is:



The **initiation** phase in a radical chain reaction involves the homolytic cleavage of a weak single bond in a non-radical compound, resulting in two radical species as products. Often, heat or light provides the energy necessary to overcome an energy barrier for this type of event. The initiation step in alkane halogenation is homolysis of molecular chlorine (Cl₂) into two chlorine radicals. Keep in mind that that virtually all radical species, chlorine radicals included, are highly reactive.

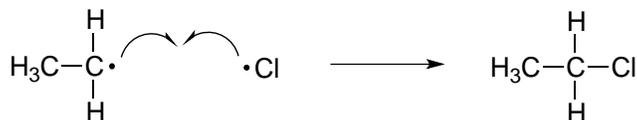


The **propagation** phase is the 'chain' part of chain reactions.



Once a reactive free radical (chlorine radical in our example) is generated in the initiation phase, it will react with relatively stable, non-radical compounds to form a new radical species. In ethane halogenation, a chlorine radical generated in the initiation step first reacts with ethane in a hydrogen abstraction step, generating HCl and an ethyl radical (part a above). Then, the ethyl radical reacts with another (non-radical) Cl₂ molecule, forming the chloroethane product and regenerating a chlorine radical (part b above). This process repeats itself again and again, as chlorine radicals formed in part (b) react with additional ethane molecules as in part (a).

The **termination** phase is a **radical combination** step, where two radical species happen to collide and react with each other to form a non-radical product and 'break the chain'. In our ethane chlorination example, one possible termination event is the reaction of a chlorine radical with an ethyl radical to form chloroethane.

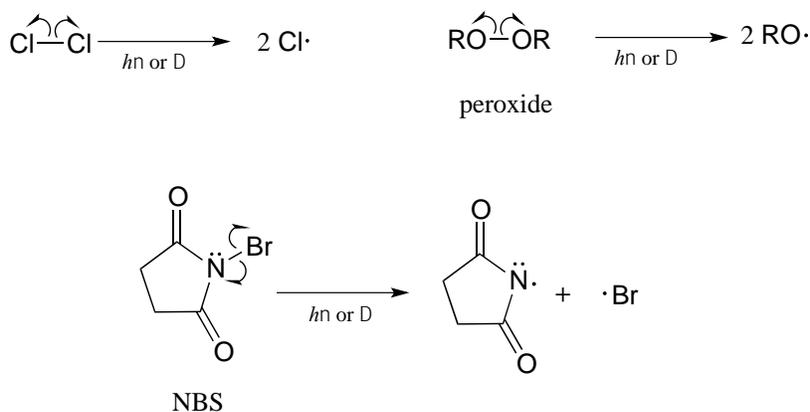


Exercise 16.3: Draw two alternative chain termination steps in the ethane chlorination chain reaction. Which one leads to an undesired product?

Because radical species are so reactive and short-lived, their concentration in the reaction mixture at any given time is very low compared to the non-radical components such as ethane and Cl₂. Thus, many cycles of the chain typically occur before a termination event

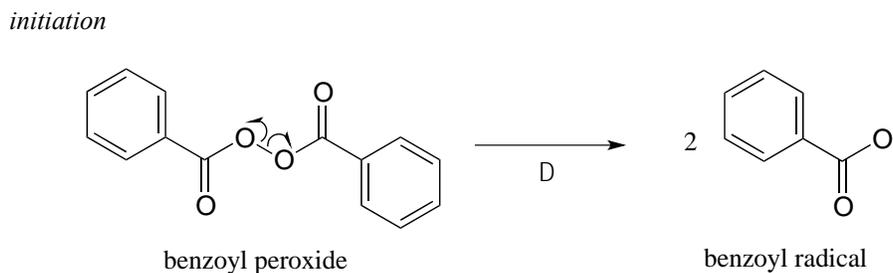
takes place. In other words, a single initiation event leads to the formation of many product molecules.

Compounds which readily undergo homolytic cleavage to generate radicals are called **radical initiators**. As we have just seen, molecular chlorine and bromine will readily undergo homolytic cleavage to form radicals when subjected to heat or light. Other commonly used as radical initiators are peroxides and *N*-bromosuccinimide (NBS).

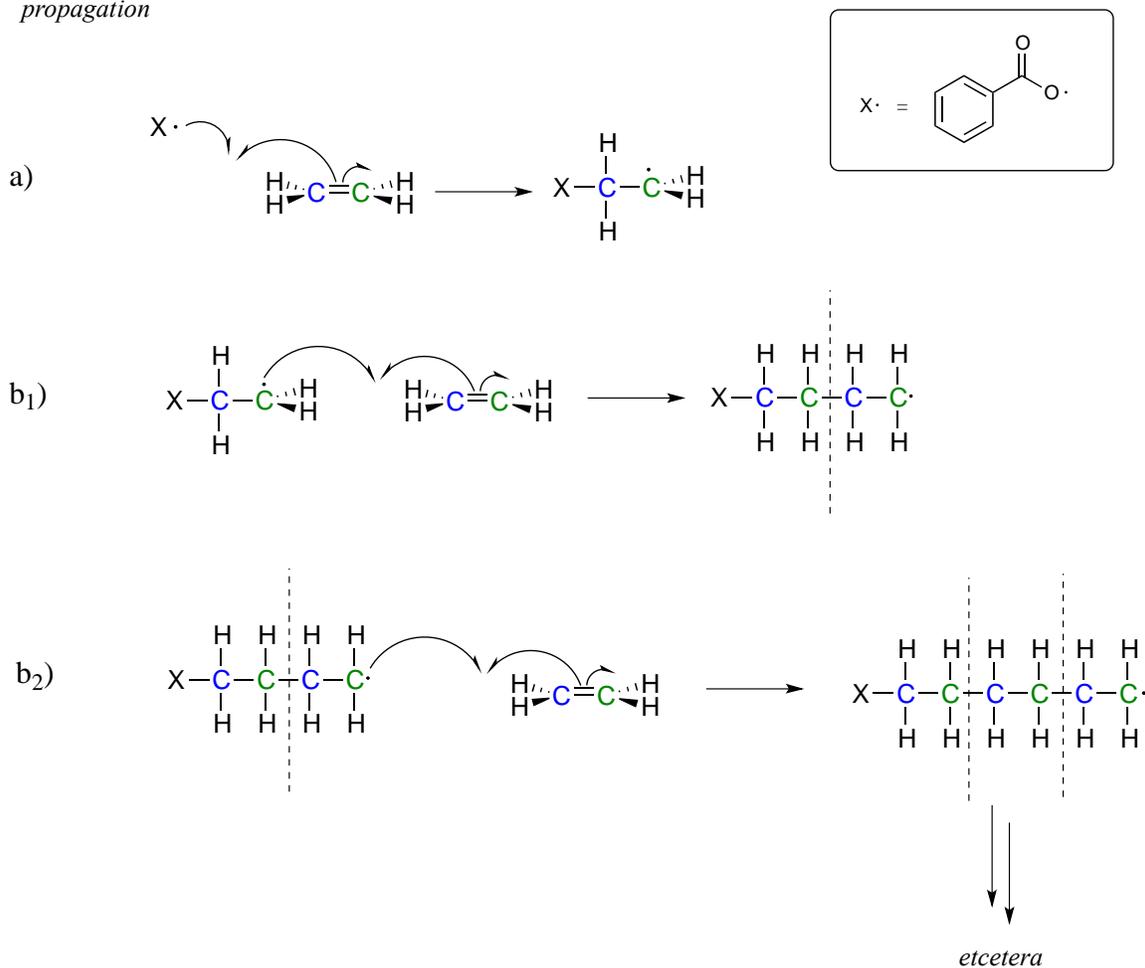


Section 16.3: Useful polymers formed by radical chain reactions

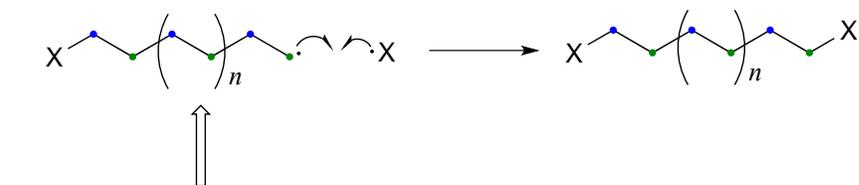
Many familiar household materials polymers made from radical chain reaction processes. Polyethylene (PET), the plastic material used to make soft drink bottles and many other kinds of packaging, is produced by the radical polymerization of ethylene (ethylene is a common name for what we call 'ethene' in IUPAC nomenclature). The process begins when a radical initiator such as benzoyl peroxide undergoes homolytic cleavage at high temperature:



In the propagation phase, the benzoyl radical ($X\cdot$ in the figure below) adds to the double bond of ethylene, generating a new organic radical.

propagation

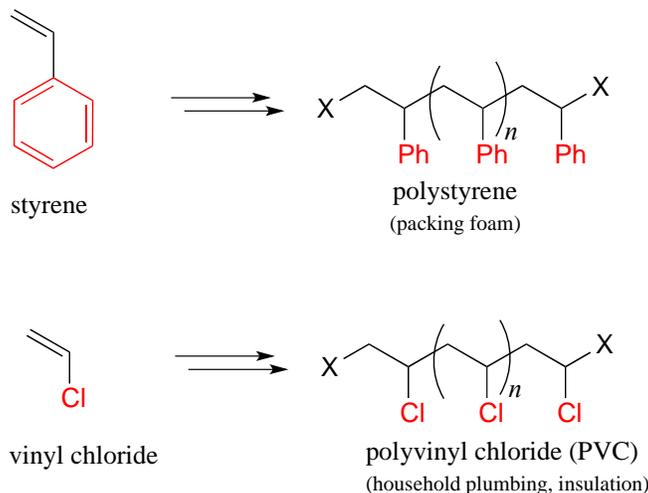
Successive ethylene molecules add to the growing polymer, until termination occurs when two radicals happen to collide.

termination

this notation indicates that there are many repeating $-CH_2CH_2-$ monomers in the chain

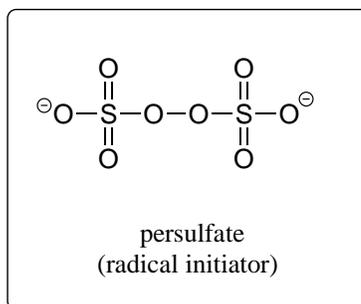
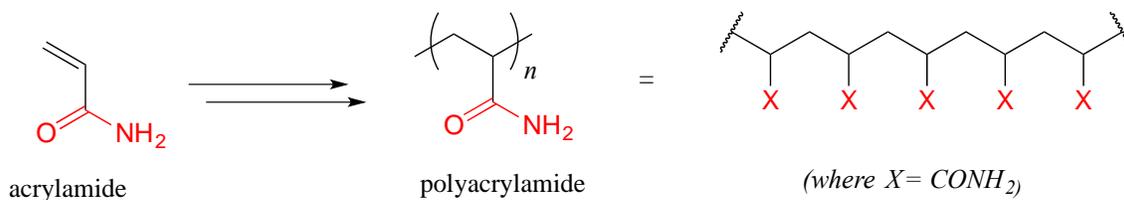
The length of the polymer is governed by how long the propagation phase continues before termination, and can usually be controlled by adjusting reaction conditions.

Other small substituted alkene monomers polymerize in a similar fashion to form familiar polymer materials. Two examples are given below.



Exercise 16.4: Show a mechanism for the formation of a 2-unit long section of polystyrene, starting with the monomer and benzoyl peroxide initiator. Keep in mind the relative stability of different radical intermediates.

A common way to separate proteins in the biochemistry lab is through a technique called polyacrylamide gel electrophoresis (PAGE). The polyacrylamide gel is formed through radical polymerization of acrylamide monomer, with the ammonium salt of persulfate used as the radical initiator.

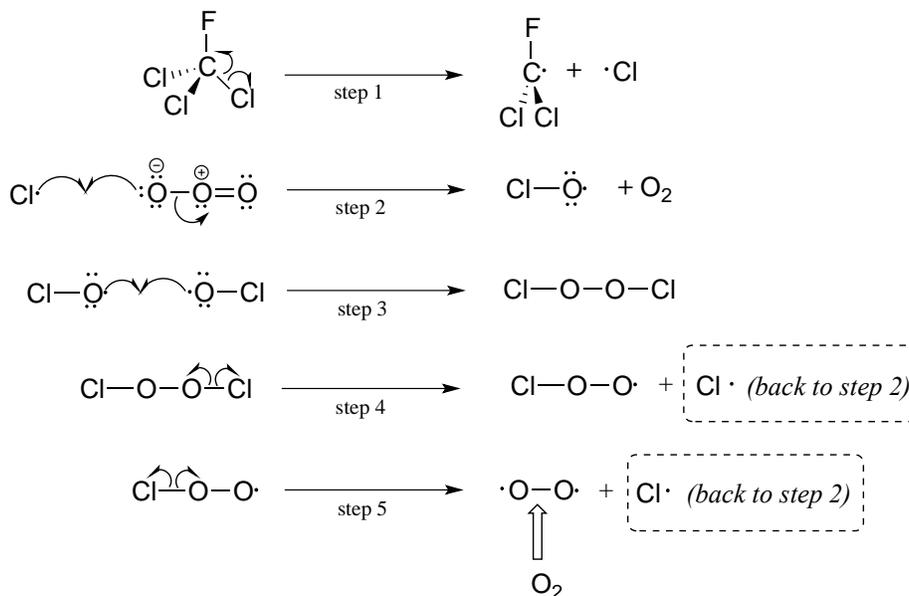


In the end of chapter problems, you will be invited to propose a mechanism showing how a molecule called '*bis*-acrylamide' serves as a 'crosslinker' between linear polyacrylamide chains to allow for formation of a net-like structure for the PAGE gel.

Section 16.4: Destruction of the ozone layer by a radical chain reaction

The high reactivity of free radicals and the multiplicative nature of radical chain reactions can be useful in the synthesis of materials such as polyethylene plastic - but these same factors can also result in dangerous consequences in a biological or ecological context. You are probably aware of the danger posed to the earth's protective stratospheric ozone layer by the use of chlorofluorocarbons (CFCs) as refrigerants and propellants in aerosol spray cans. Freon-11, or CFCl_3 , is a typical CFC that was widely used until late in the 20th century. It can take months or years for a CFC molecule to drift up into the stratosphere from the surface of the earth, and of course the concentration of CFCs at this altitude is very low. Ozone, on the other hand, is continually being formed in the stratosphere. Why all the concern, then, about destruction of the ozone layer - how could such a small amount of CFCs possibly do significant damage? The problem lies in the fact that the process by which ozone is destroyed is a *chain* reaction, so that a single CFC molecule can initiate the destruction of many ozone molecules before a chain termination event occurs.

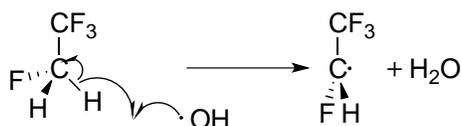
Although there are several different processes by which the ozone destruction process might occur, the most important is believed to be the chain reaction shown below.



First, a CFC molecule undergoes homolytic cleavage upon exposure to UV radiation, resulting in the formation of two radicals (step 1). The chlorine radical rapidly reacts

with ozone (step 2) to form molecular oxygen and a chlorine monoxide radical. Step 3 *appears* to be a chain termination step, as two chlorine monoxide radicals combine. The Cl_2O_2 condensation product, however, is highly reactive and undergoes two successive homolytic cleavage events (steps 4 and 5) to form O_2 and two chlorine radicals, which propagates the chain.

To address the problem of ozone destruction, materials chemists have developed new hydrofluorocarbon refrigerant compounds. The newer compounds contain carbon-hydrogen bonds, which are weaker than the carbon-halogen bonds in CFCs, and thus are susceptible to homolytic cleavage caused by small amounts of hydroxide radical present in the lower atmosphere:



This degradation occurs *before* the refrigerant molecules have a chance to drift higher up to the stratosphere where the ozone plays its important protective role. The degradation products are quite unstable and quickly degrade further into relatively harmless by-products. The hydroxide radical is sometimes referred to as an atmospheric 'detergent' due to its ability to degrade escaped refrigerants and other volatile organic pollutants.

Hydrofluorocarbons do, however, act as greenhouse gases, and are thought to contribute to climate change.

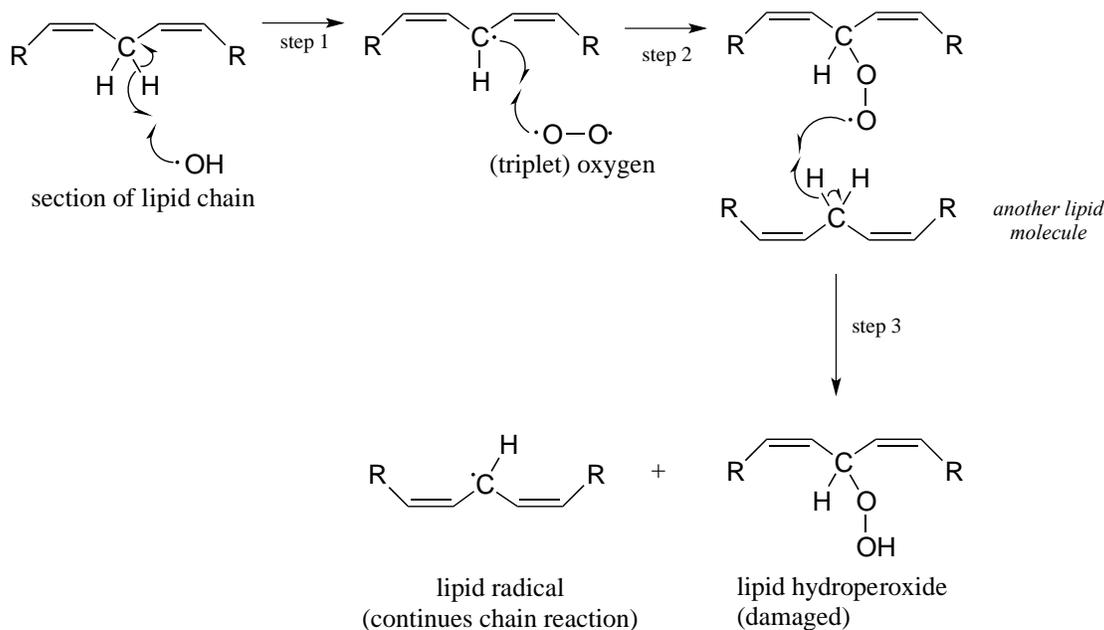
Section 16.5: Oxidative damage, vitamin C, and scurvy

While the hydroxide radical can be a beneficial 'detergent' in the atmosphere, it is harmful when present in a living cell. Hydroxide radical is one of the reactive oxygen species (ROS) that we learned about in chapter 15. Recall that ROS are continuously produced as minor but harmful side-products in the reduction of O_2 to H_2O in respiration.

You may recall from your general chemistry course that molecular oxygen exists in two states: 'singlet' oxygen has a double bond and no unpaired electrons, while 'triplet' oxygen has a single O-O bond and two unpaired electrons. Molecular orbital theory - and experimental evidence - show that the triplet state is lower in energy.

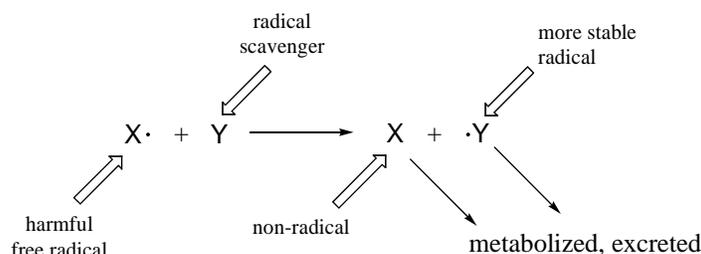


ROS are highly reactive oxidizing agents, capable of inflicting damage to DNA, proteins, and the lipids of cell membranes - they are thought to play a major role in the aging process. Hydroxide radical, for example, can initiate a radical chain reaction with the hydrocarbon chain of an unsaturated membrane lipid molecule, resulting in the formation of lipid hydroperoxide.



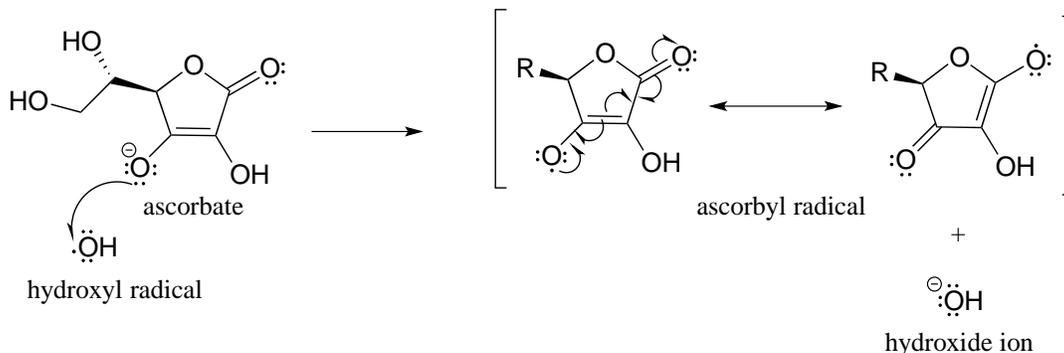
The allylic lipid radical formed as the result of homolytic hydrogen abstraction by hydroxide radical (step 1 above) reacts with one of the unpaired electrons in triplet oxygen (step 2) forming a peroxy radical. This radical species in turn homolytically abstracts a hydrogen from another lipid molecule (step 3), thus propagating the chain.

Many edible plants contain various antioxidant compounds, also known as 'free radical scavengers', which serve to protect cells from the oxidative effects of hydroxide radical and other harmful radical intermediates. Simply put, a free radical scavenger is a molecule that reacts with a potentially damaging free radical species, forming a *more stable* radical species which can be metabolized by the body before any damage is done to cell constituents.



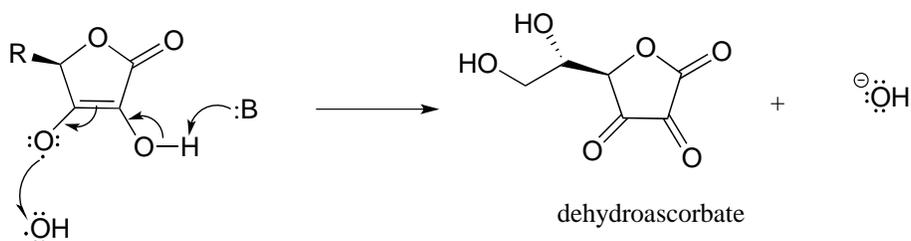
In the introduction to this chapter, we learned about scurvy, the disease long dreaded by sailors, and how it is caused by a deficiency of ascorbic acid (vitamin C) in the diet. We will soon get to the connection between ascorbic acid and scurvy, but first, let's look at how ascorbic acid functions as a free radical scavenger in your body.

The pK_a of ascorbic acid is about 4.1, so in a physiological environment it exists mainly as ascorbate anion, the conjugate base. When ascorbate encounters a hydroxyl radical (or any other potentially damaging radical species), it donates a single electron, thus reducing the hydroxyl radical to hydroxide ion and becoming itself an ascorbyl radical.



The ascorbyl radical is stabilized by resonance. The end result of this first step is that a very reactive, potentially harmful hydroxyl radical has been 'quenched' to hydroxide ion and replaced by a much less reactive (and thus less harmful) ascorbyl radical.

The ascorbyl radical can then donate a second electron to quench a second hydroxyl radical, resulting in the formation of dehydroascorbate, the oxidized form of ascorbate.



One ascorbate molecule is thus potentially able to scavenge *two* harmful radical species.

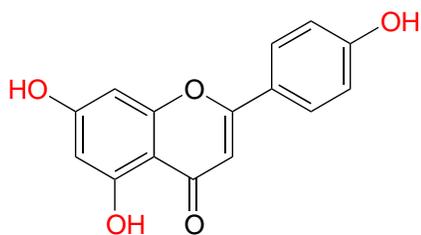
Dehydroascorbate is subsequently either broken down and excreted, or else enzymatically recycled (reduced) back to ascorbic acid. You were invited to propose a mechanism for the latter (redox) step in problem 15.10.

We learned in the introduction to this chapter about the gruesome effects of long-term ascorbic acid deficiency. What, then, is the chemical connection between ascorbic acid and scurvy?

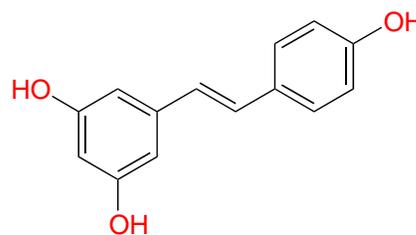
chemistry course. It is enough for us to know that iron starts out in the Fe^{+2} state, and during the course of the reaction it loses an electron to assume the Fe^{+3} state. In order for the enzyme to catalyze another reaction, the iron must be reduced back to its active Fe^{+2} state - it must accept a single electron. The donor of this single electron is ascorbate. (For more information, see *Crit. Rev. Biochem. Mol. Biol.* **2010**, 405, 106.)

So, to sum up: If we fail to get enough ascorbic acid in our diet (in other words, if we don't eat our fruits and vegetables!) the iron in our proline hydroxylase enzymes won't be returned to the active Fe^{+2} state, so the catalytic cycle is broken and we can't turn prolines into hydroxyprolines. Without the hydroxy group, the proline residues of our collagen proteins won't assume the proper conformation, and as a consequence the collagen triple helix structures will be unstable. At physiological temperature, our collagen will literally melt apart - and with it, our gums, our capillaries, and anything else held together by connective tissue. This is scurvy.

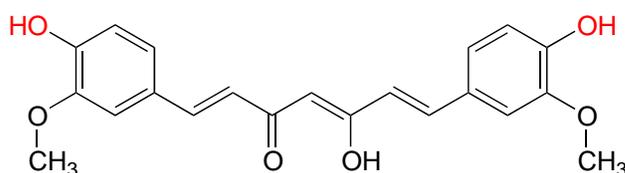
You have probably heard that many fruits and vegetables contain natural 'polyphenol' antioxidant compounds that are thought to be beneficial to our health. Apigenin, for example, is found in parsley and celery, while the skins of grapes used to produce red wine are particularly rich in resveratrol, as well as many other polyphenols. Curcumin is the compound responsible for the distinctive yellow color of turmeric, a ubiquitous spice in Indian cuisine.



apigenin

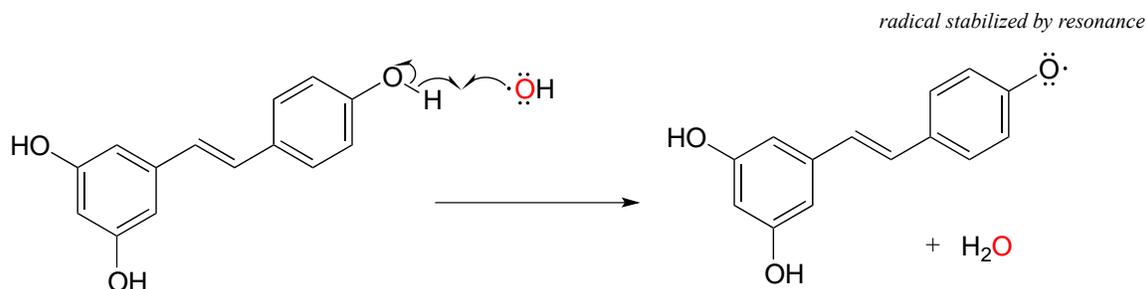


resveratrol



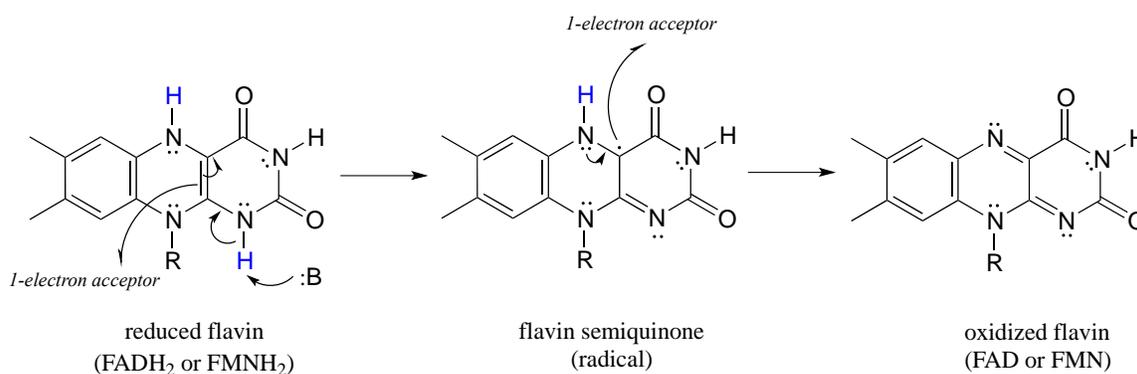
curcumin

While much remains to be learned about exactly how these polyphenols exert their antioxidant effects, it is likely that they, like ascorbic acid, act as radical scavengers. For example, resveratrol could donate a single electron (and a proton) to hydroxide radical to reduce it to water. The phenolic radical that results is stabilized by resonance, and is much less likely than hydroxide radical to cause damage to important biomolecules in the cell.



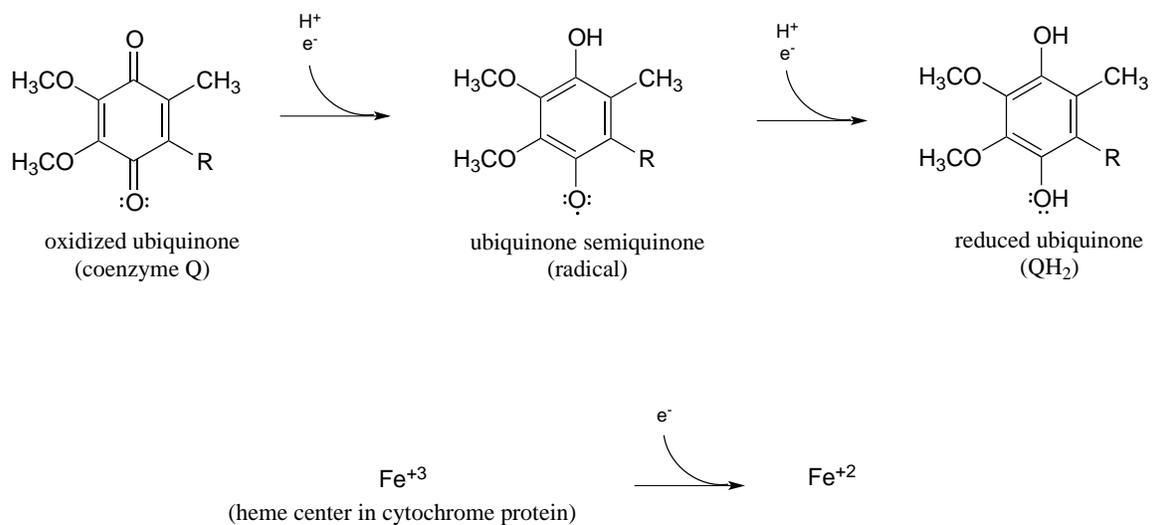
Section 16.6: Flavin as a single-electron carrier

In chapter 15 we saw how a nicotinamide and flavin coenzymes can act as acceptors or donors of *two* electrons in hydride-transfer redox steps. Recall that it was mentioned that flavin, (but *not* nicotinamide) can also participate in *single-electron* transfer steps through a stabilized radical intermediate called a **semiquinone**.



Note in this reaction that overall, flavin loses or gains two electrons and two protons, just like in the flavin-dependent redox reactions we saw in chapter 15. The difference here is that the electrons are transferred *one at a time*, rather than paired in the form of a hydride ion.

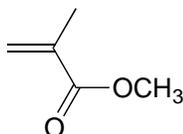
Two important examples single-electron acceptor species in human metabolism are ubiquinone (coenzyme Q) and the oxidized form of cytochrome. Ubiquinone is a coenzyme that can transfer single electrons via a semiquinone state analogous to that of flavin, and cytochrome is a protein containing a 'heme' iron center which shuttles between the Fe⁺³ (oxidized) and Fe⁺² (reduced) state.



Further discussion of the mechanisms of single-electron flavin reactions is beyond our scope here, but when you study the 'respiratory chain' in a biochemistry course you will gain a deeper appreciation for the importance of flavin in single-electron transfer processes.

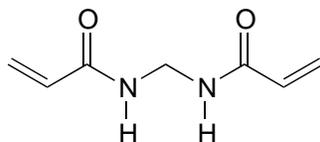
Problems

P16.1: Plexiglass is a polymer of methyl methacrylate. Show a mechanism for the first two propagation steps of polymerization (use $X\cdot$ to denote the radical initiator), and show a structure for the plexiglass polymer. Assume an alkene addition process similar to that shown in the text for polyethylene.



methyl methacrylate

P16.2: In section 16.3 we saw how acrylamide polymerizes to form the polyacrylamide used in PAGE protein gels. Polyacrylamide by itself is not sufficient by itself to form the gel - the long polyacrylamide chains simply slip against each other, like boiled spaghetti. To make a PAGE gel, with pores for the proteins to slip through, we need a crosslinker - something to tie the chains together, forming a three-dimensional web-like structure. Usually, a small amount of *bis*-acrylamide is added to the acrylamide in the polymerization mixture for this purpose.



bis-acrylamide

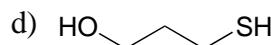
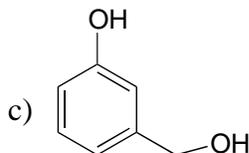
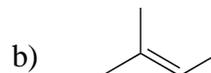
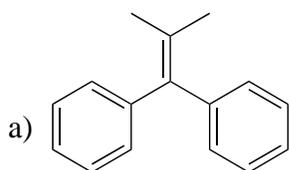
Propose a radical mechanism showing how *bis*-acrylamide might form crosslinks between two polyacrylamide chains.

P16.3: Resveratrol is a natural antioxidant found in red wine (see section 16.5 for the structure).

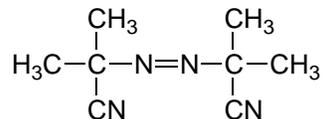
- Draw one resonance structure to illustrate how the resveratrol radical is delocalized by resonance.
- Indicate all of the carbons on your structure to which the radical can be delocalized.
- Draw an alternate resveratrol radical (one in which a hydrogen atom from one of the other two phenolic groups has been abstracted). To how many carbons can this radical be delocalized?

d) The curcumin structure is shown in the same figure as that of resveratrol, in section 16.5. Draw two resonance contributors of a curcumin radical, one in which the unpaired electron is on a phenolic oxygen, and one in which the unpaired electron is on a ketone oxygen.

P16.4: Draw the radical intermediate species that you would expect to form when each of the compounds below reacts with a radical initiator.



P16.5: Azobis(isobutyronitrile) is a widely used radical initiator which rapidly undergoes homolytic decomposition when heated.



Azobis(isobutyronitrile)

Predict the products of this decomposition reaction, and show a likely mechanism. What is the thermodynamic driving force for homolytic cleavage?

P16.6:

a) When 2-methylbutane is subjected to chlorine gas and heat, a number of isomeric chloroalkanes with the formula $\text{C}_5\text{H}_{11}\text{Cl}$ can form. Draw structures for these isomers, and for each draw the alkyl radical intermediate that led to its formation.

b) In part a), which is the most stable radical intermediate?

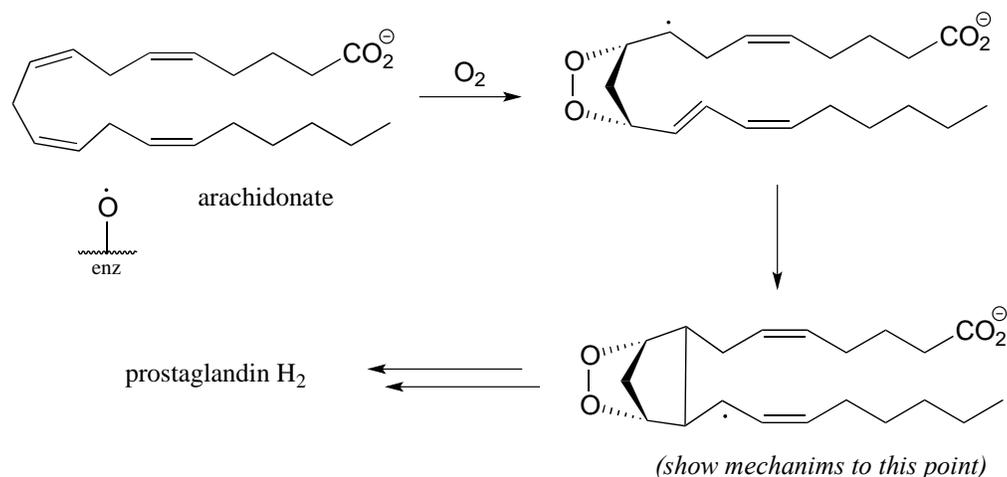
c) In the reaction in part a), the relative abundance of different isomers in the product is not *exclusively* a reflection of the relative stability of radical intermediates. Explain.

P16.7: We learned in chapter 14 that HBr will react with alkenes in electrophilic addition reactions with 'Markovnikov' regioselectivity. However, when the starting alkene contains even a small amount of contaminating peroxide (which happens when it is allowed to come into contact with air), a significant amount of 'anti-Markovnikov' product is often observed.

a) Propose a mechanism for formation of the anti-Markovnikov addition product when 1-butene reacts with HBr containing a small amount of benzoyl peroxide

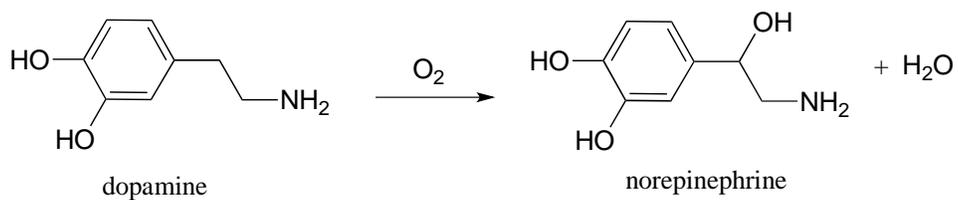
b) Predict the product and propose a mechanism for the addition of ethanethiol to 1-butene in the presence of peroxide.

P16.8: In section 11.5 we learned that aspirin works by blocking the action of an enzyme that catalyzes a key step in the biosynthesis of prostaglandins, a class of biochemical signaling molecules. The enzyme in question, prostaglandin H synthase (EC 1.14.99.1) catalyzes the reaction *via* several single-electron steps. First, an iron-bound oxygen radical in the enzyme abstracts a hydrogen atom from arachidonate. The arachidonate radical intermediate then reacts with molecular oxygen to form a five-membered oxygen-containing ring, followed by closure of a cyclopentane ring to yield yet another radical intermediate.

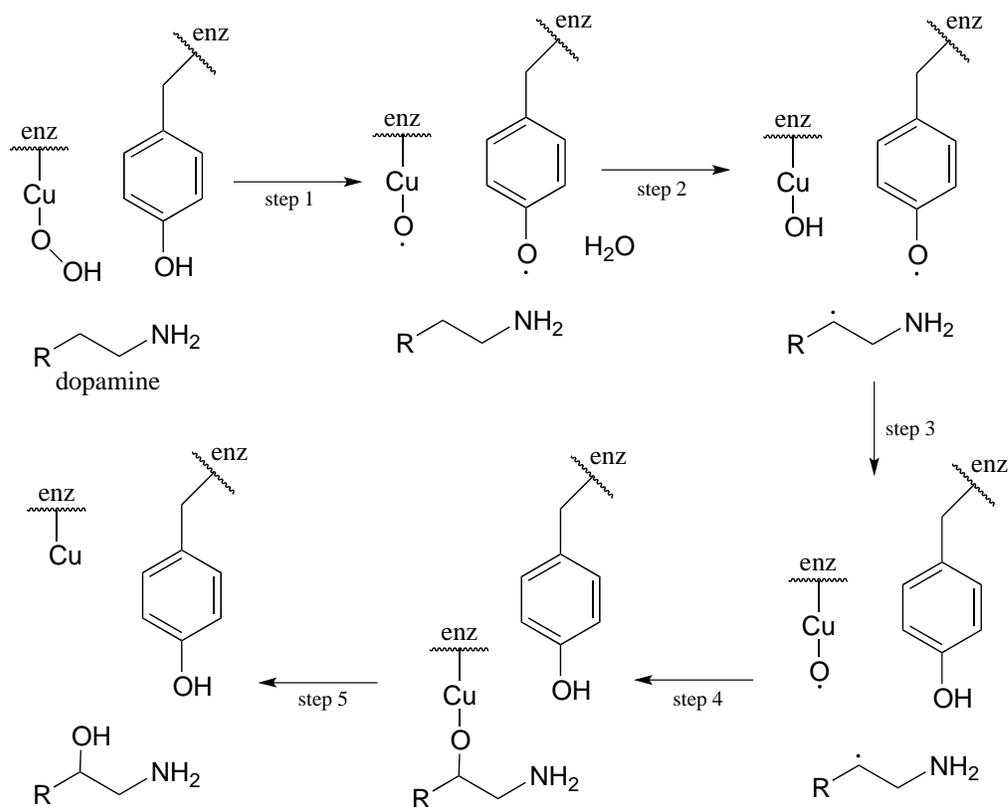


Propose a mechanism for the steps of the reaction that are shown in this figure.

P16.9: Some redox enzymes use copper to assist in electron transfer steps. One important example is dopamine β -monooxygenase (EC 1.14.1.1), which catalyzes the following reaction:



The following intermediates have been proposed: (see *Biochemistry* **1994**, 33, 226)



Draw mechanistic arrows for steps 1-4.

Chapter 17

Coenzymes

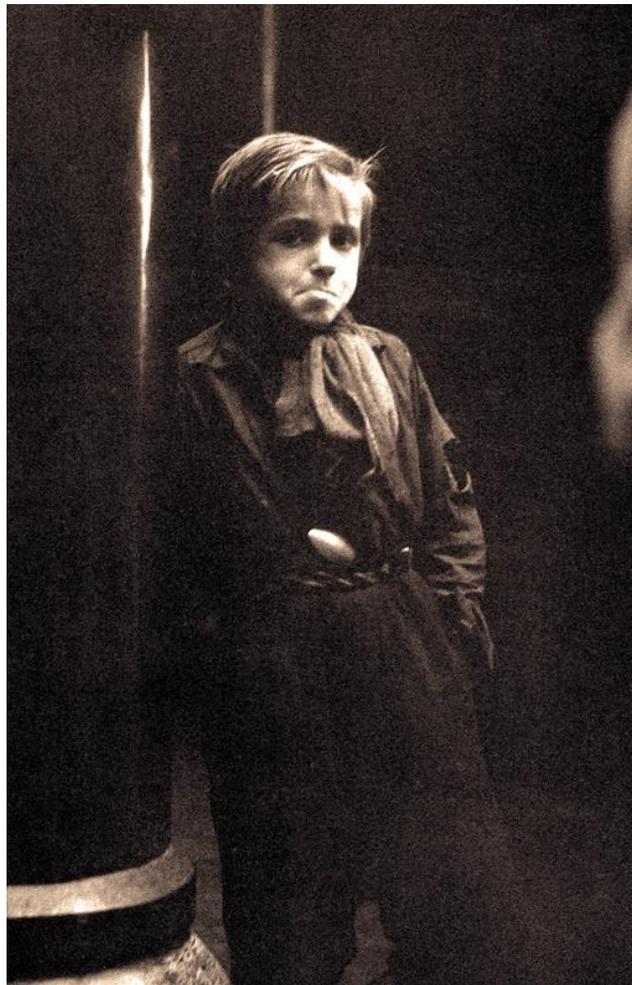


Photo: Taconis Kryn, Library and Archives Canada - PA169941

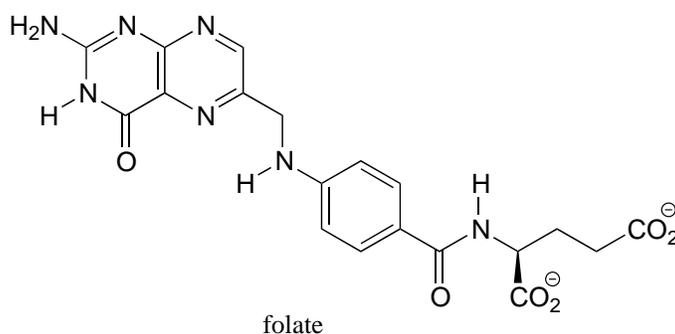
The old black and white photograph is haunting. A young boy, perhaps 10 or 11 years old, huddles against a wall outside a soup kitchen, his mouth in an odd twisted shape that

could be expressing either pain or defiance, his eyes staring straight into those of the viewer. Tucked into his pants, almost like a pistol in a holster, is a metal spoon.

The photograph was taken in the Netherlands in 1945, at the height of what the Dutch people still refer to simply as "The Hunger Winter". With the western part of the country still occupied by the Nazis, the Dutch resistance government, based in London, had called for a railway strike with the aim of stopping German troop movements before a planned airborne invasion by Allied forces. In retaliation, the Germans cut off all food shipments to cities in the western Netherlands. The Allied invasion failed to liberate the country, and the winter of 1944-1945 turned out to be bitterly cold. With food supplies dwindling, rations were cut first to 1000 calories per day, then to 500. People resorted to eating grass and tulip bulbs just to stay alive. Over 20,000 people died of starvation before food shipments were restored in the spring.

As tragic as the Hunger Winter was for the Dutch people, some good did come from it. For medical scientists, the event became a unique 'social experiment': unlike most episodes of famine throughout human history, the Hunger Winter had a clearly defined beginning, end, and geographic boundary, and it occurred in a technologically advanced society that continued to keep thorough records before, during, and after the ordeal. Scientists knew exactly who suffered from famine and for precisely how long, and in the years that followed they were able to look at the long-term effects of famine, particularly on developing embryos. Researchers found that babies who had been conceived during the famine were born with a significantly higher incidence of neurological birth defects such as spina bifida, a condition in which a portion of the neural tube protrudes from between vertebrae which did not properly fuse together during fetal development. Later in life, members of this same cohort of 'famine babies' were more likely to be obese, and to suffer from schizophrenia.

These initial findings spurred interest in further research into the consequences of prenatal deprivation. In particular, carefully controlled studies later led to the recognition of the importance of the vitamin folate in ensuring proper neurological development in early-term fetuses.



Folate - the conjugate base of folic acid - is an organic **coenzyme**: a helper molecule that binds in the active site of certain enzymes and plays a critical role in the biochemical reaction being catalyzed. Recall that we have seen coenzymes at work before: SAM,

ATP, NAD(P)^+ and NAD(P)H , flavin and glutathione are all important coenzymes with which we are already familiar.

Because prenatal folate deficiency was found to be directly related to the incidence of spina bifida and other neural tube defects, health officials in the United States and many other countries changed their official guidelines to include a specific recommendation that women begin taking folate supplements as soon as they knew that they are pregnant, or better yet as soon as they begin trying to become pregnant. A number of studies conducted during the 1980s and early 1990s consistently showed that folate supplementation correlated with a 50-70% reduction in neural tube defects.

The molecular role of folate in prenatal neurological development is not understood in detail, but most researchers agree that it probably has a lot to do with DNA biosynthesis. Like *S*-adenosyl methionine (SAM), folate functions in 1-carbon transfer reactions, including several critical steps in the nucleic acid biosynthesis pathways. The rapidly dividing cells of the developing brain of an early term fetus appear to be especially sensitive to folate deficiency in the mother's diet: insufficient folate leads to impaired DNA biosynthesis, which in turn leads to defects in brain development.

Folate also serves as a 1-carbon donor in the pathway by which SAM is regenerated after it donates a methyl group. You may recall from the introduction to chapter 8 that methylation of cytosine bases in DNA by SAM results in permanent changes to an individual's genome - this was the reason why the two 'identical' twin sisters in that introductory story turned out to be, as they grew older, not so identical after all. It is likely that the folate deprivation that afflicted expectant mothers during the Dutch Hunger Winter also caused epigenetic changes (in other words, changes in the extent of DNA methylation) in their developing fetuses, which decades later manifested in the form of an increased incidence of conditions such as obesity and schizophrenia. All the more reason, we now know, to make sure that women get plenty of folate in their diet early in the first trimester of pregnancy.

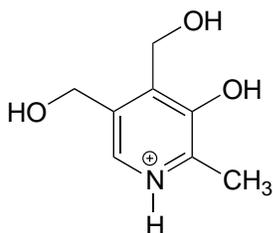
In this final chapter, we focus on the organic chemistry of folate, along with three other coenzymes: pyridoxal phosphate, thiamine diphosphate, and lipoamide. Humans can synthesize lipoamide, but we depend on dietary sources for the other three: pyridoxal phosphate is a form of vitamin B₆, and thiamine diphosphate is a form of vitamin B₁. In a mechanistic sense, there is really nothing new in this chapter. All of the reaction mechanism types that we will see are already familiar to us, ranging from nucleophilic substitutions (chapter 8) to disulfide exchanges (chapter 15). We will soon see, however, how each coenzyme plays its own specific and crucial role in assisting enzymes with the catalysis of key reactions of metabolism. We will begin with pyridoxal phosphate and its various roles in amino acid metabolism.

Additional reading:

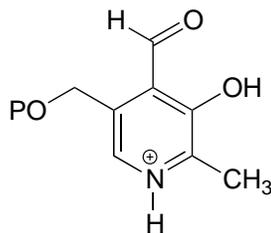
<http://www.naturalhistorymag.com/features/142195/beyond-dna-epigenetics>

Section 17.1: Pyridoxal phosphate (Vitamin B₆)

The coenzyme pyridoxal phosphate (commonly abbreviated PLP) is the active form of vitamin B₆, or pyridoxine.



pyridoxine (vitamin B₆)

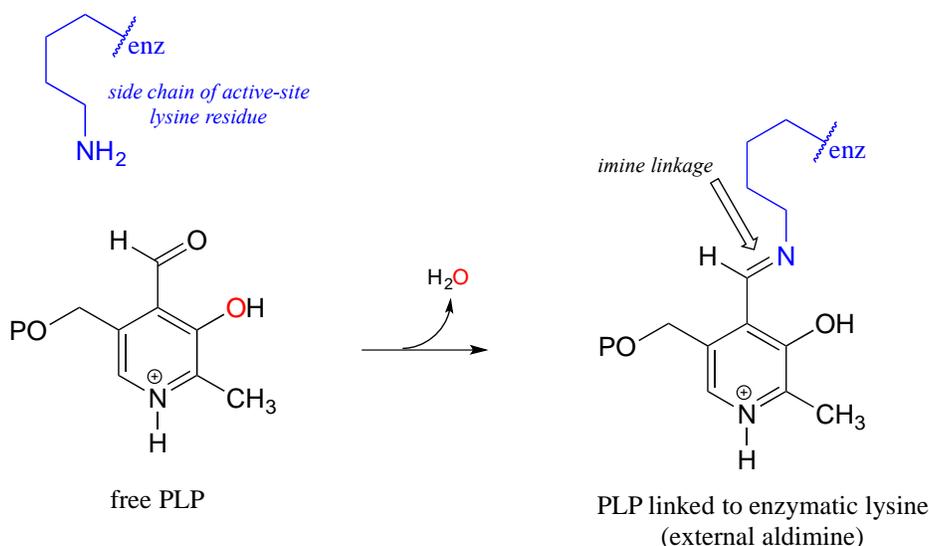


pyridoxal phosphate (PLP)

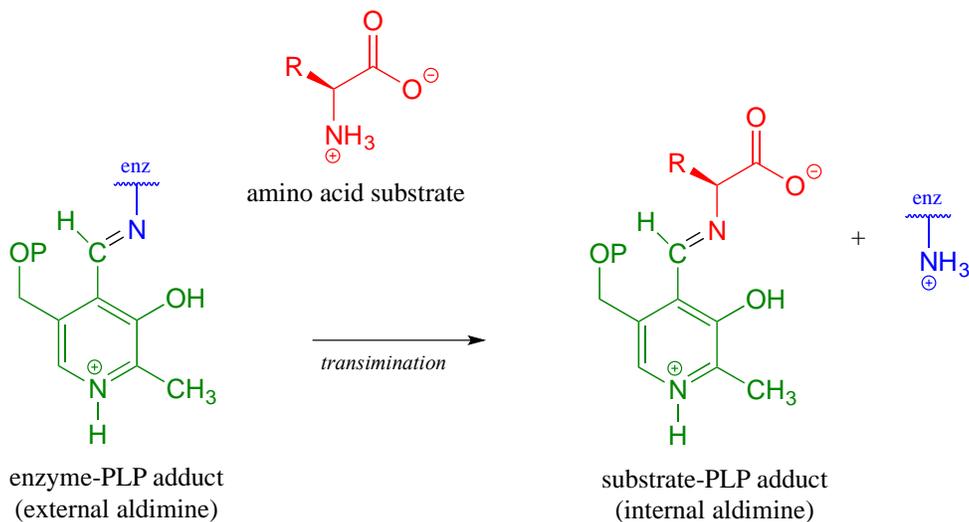
PLP is required for over 100 different reactions in human metabolism, primarily in the various amino acid biosynthetic and degradation pathways. The essential function of PLP is to act as an 'electron sink', stabilizing a negative formal charge that develops on key reaction intermediates. Some of reactions will be familiar to you from chapter 12 and 13: we will see examples, for instance, of PLP-dependent α -carbon racemization, as well as aldol- and Claisen-type reactions. Other reactions will be less familiar: for example, the participation of PLP allows for decarboxylation of amino acids, a chemical step which would be highly unlikely without the coenzyme, and PLP is also required for a very important class of biochemical transformation called 'transamination', in which the amino group of an amino acid is transferred to an acceptor molecule. Before we dive into the reactions themselves, though, we need to begin by looking at a key preliminary step that is common to all of the PLP reactions we will see in this section.

17.1A: PLP in the active site: the imine linkage

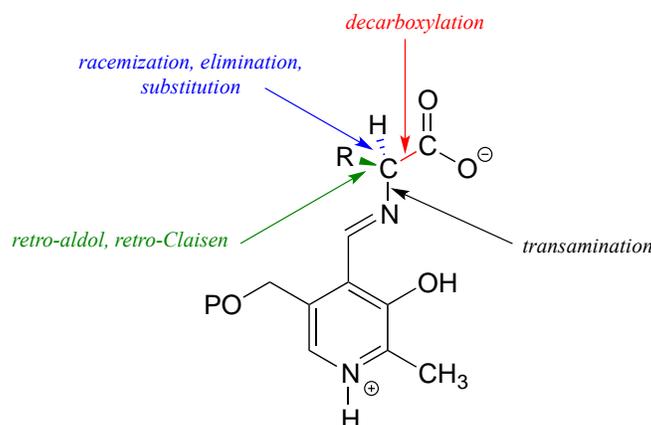
The common catalytic cycle of a PLP-dependent enzyme begins and ends with the coenzyme *covalently* linked to the enzyme's active site through an imine linkage between the aldehyde carbon of PLP and the amine group of a lysine residue (see section 10.5 to review the mechanism for imine formation). For a PLP-dependent enzyme to become active, a PLP molecule must first enter the active site of an enzyme and form an imine link to the lysine. This state is often referred to as an **external aldimine**.



The first step of virtually all PLP-dependent reactions is transamination (section 10.5), as the amino group on the amino acid substrate displaces the amino group of the enzymatic lysine. This state - where the coenzyme is covalently linked to the substrate or product of the reaction - is often referred to as an **internal aldimine**.



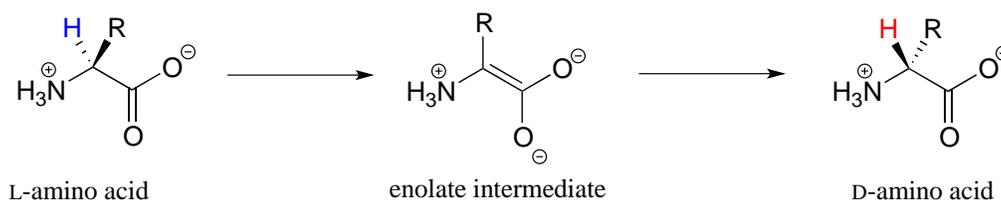
With the preliminary transamination accomplished, the real PLP chemistry is ready to start. The versatility of PLP in terms of its ability to assist with a wide variety of reaction types is illustrated by the figure below, showing how, depending upon the reaction/enzyme in question, PLP can assist in the cleavage of any one of the four bonds to the α -carbon of the amino acid substrate.



Let's look first at the reaction catalyzed by PLP-dependent alanine racemase. (EC 5.1.1.1).

17.1B: PLP-dependent amino acid racemization

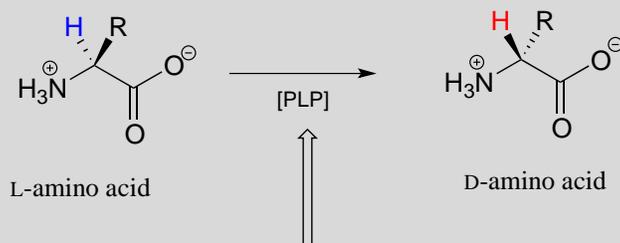
In section 12.2B we saw an example of a PLP-*independent* amino acid racemization reaction, in which the negatively-charged intermediate was simply the enolate form of a carboxylate:



Many other amino acid racemase reactions, however, require the participation of PLP.

Like all other PLP-dependent reactions that we will see in this section, PLP-dependent amino acid racemization begins with a preliminary step in which the substrate becomes attached to the coenzyme through a transamination. Once it is linked to PLP in the active site, the α -proton of an amino acid substrate is abstracted by an active site base (step 1 below). The negative charge on the carbanion intermediate can, of course, be delocalized to the carboxylate group. The PLP coenzyme, however, provides an expanded network of conjugated π -bonds over which the electron density can be delocalized all the way down to the PLP nitrogen. This is what we mean when we say that the job of PLP is to act as an '**electron sink**': the coenzyme is very efficient at absorbing, or delocalizing, the excess electron density on the deprotonated α -carbon of the reaction intermediate. *PLP is helping the enzyme to increase the acidity of the α -hydrogen by stabilizing the conjugate base.* A PLP-stabilized carbanion intermediate is commonly referred to as a **quinonoid intermediate**. Note that in the overall reaction equation below, PLP appears below the reaction arrow in brackets, indicating that it participates in the mechanism but is regenerated as part of the reaction cycle.

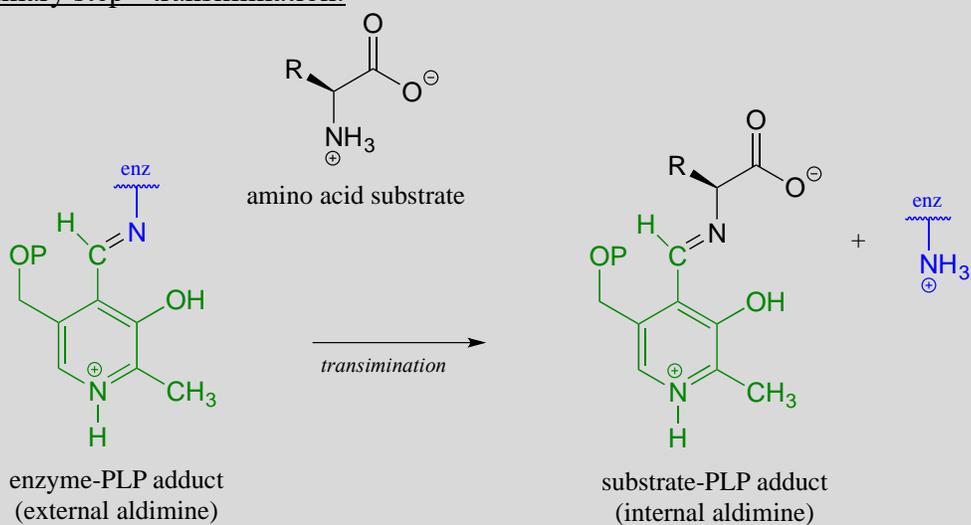
PLP-dependent amino acid racemization:



term in brackets means that the coenzyme participates in catalysis but is regenerated as part of the reaction cycle

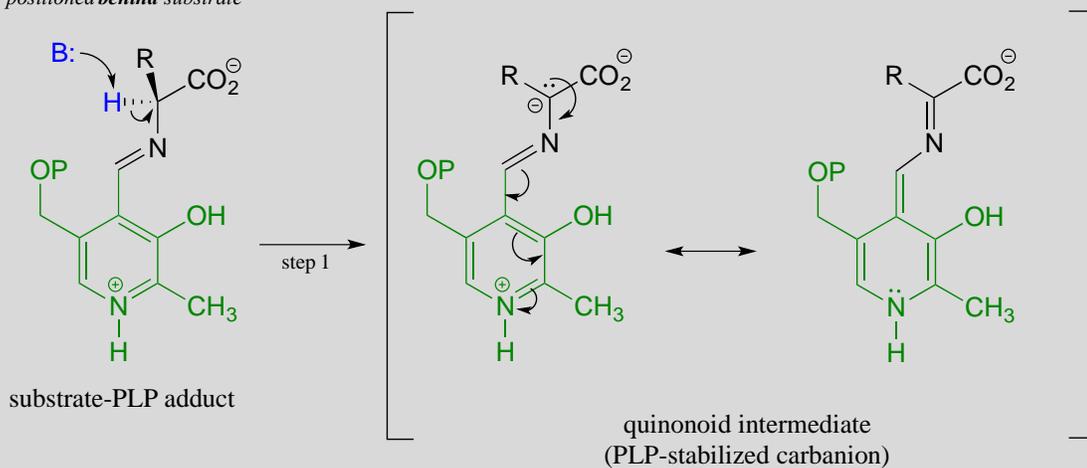
Mechanism:

Preliminary step - transimination:

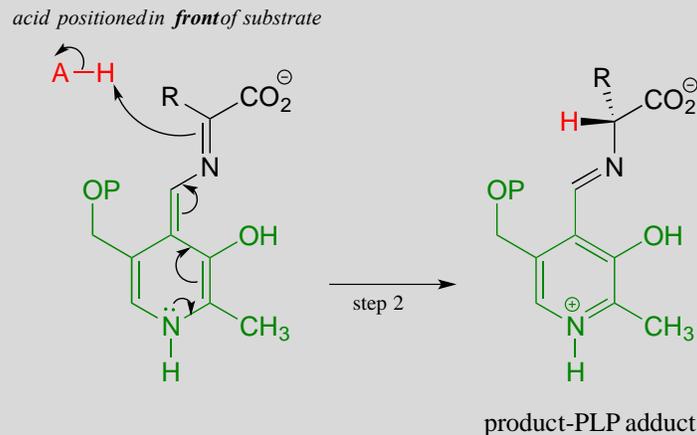


First step - deprotonation:

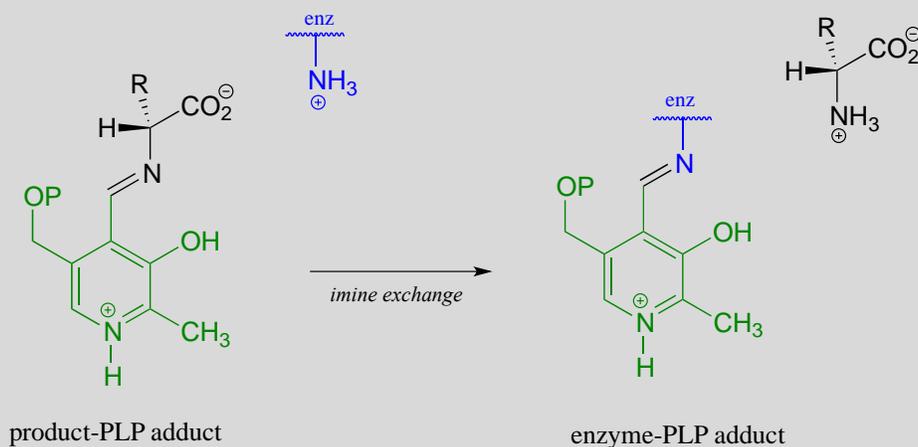
*base positioned **behind** substrate*



Second step - reprotonation from the opposite side:



Final step - transimination:



Just as in the PLP-independent racemase reactions, reprotonation occurs on the *opposite* side of the substrate (step 2), leading to the D-amino acid product.

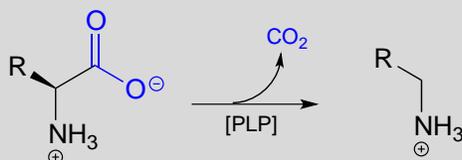
All that remains is the final imine exchange which frees the D-amino acid product and re-attaches the coenzyme to the enzymatic lysine side-chain, ready to begin another catalytic cycle.

To simplify matters, from here on we will not include the preliminary and final transimination steps in our PLP reaction figures - we will only show mechanistic steps that occur while the substrate is attached to the coenzyme (the internal aldimine forms).

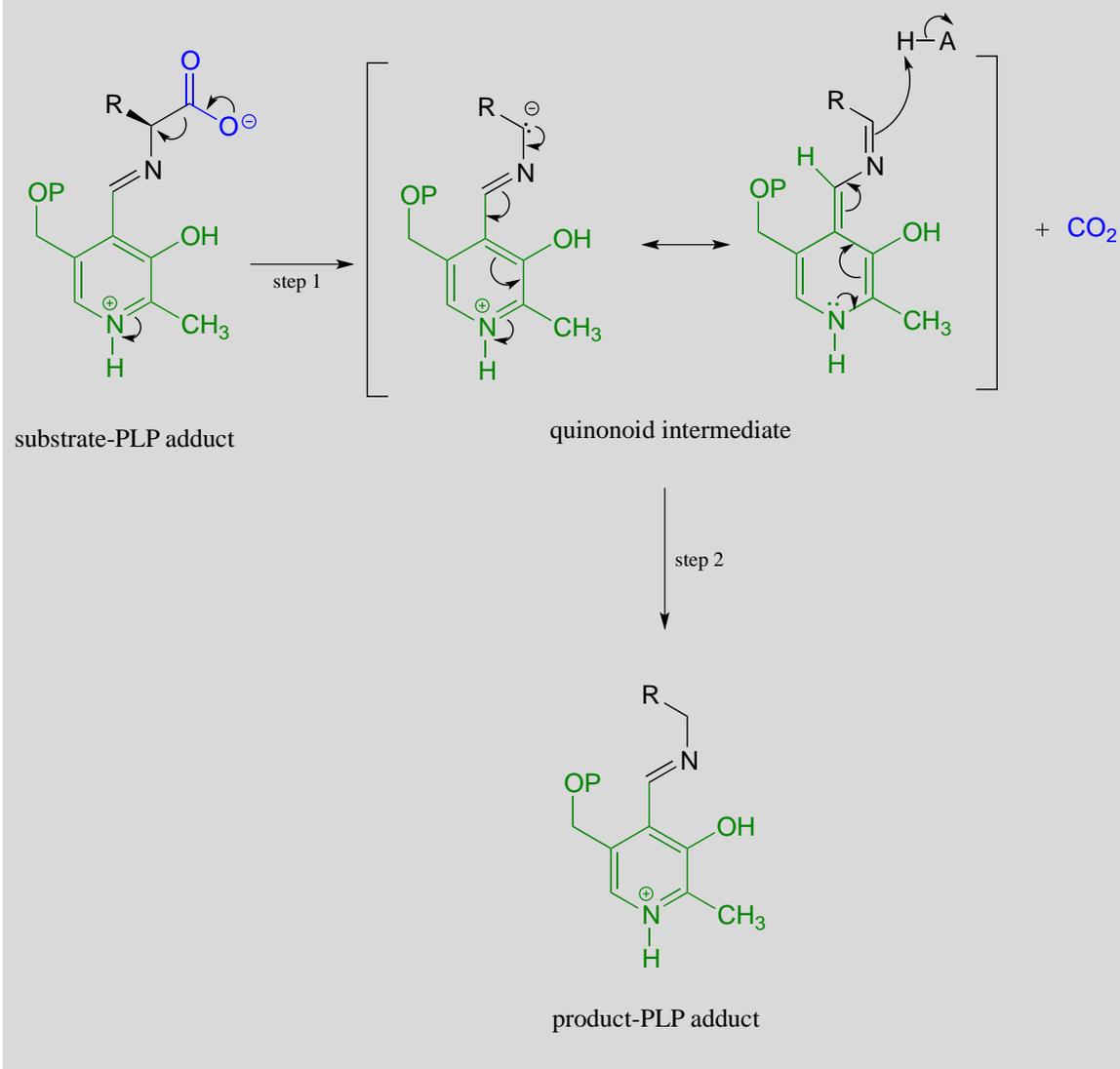
17.1C: PLP-dependent decarboxylation

In the amino acid racemase reaction above, PLP assisted in breaking the α -carbon to α -proton bond of the amino acid. Other PLP-dependent enzymes can catalyze the breaking of the bond between the α -carbon and the carboxylate carbon by stabilizing the resulting carbanion intermediate: these are simply decarboxylation reactions.

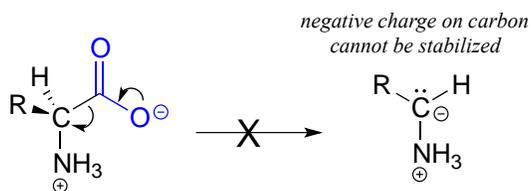
PLP-dependent amino acid decarboxylation:



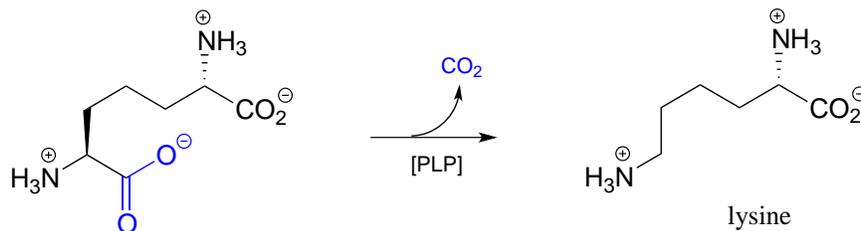
Mechanism:



Notice something very important here: while in racemization reactions the assistance of PLP can be seen as 'optional' (in the sense that some racemase enzyme use PLP and others do not), the coenzyme is *essential* for amino acid decarboxylation steps. Without PLP, there is no way to stabilize the carbanion intermediate, and decarboxylation is not a chemically reasonable step.



One example of a PLP-facilitated decarboxylation reaction is the final step in the lysine biosynthesis pathway: (EC 4.1.1.20).

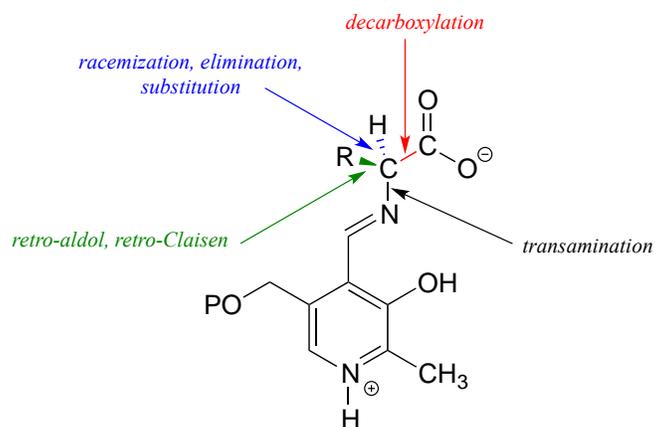


Exercise 17.1: Draw mechanistic arrows for the carbon-carbon bond-breaking step of the PLP-dependent decarboxylation reaction above.

17.1D: PLP-dependent retroaldol and retro-Claisen cleavage

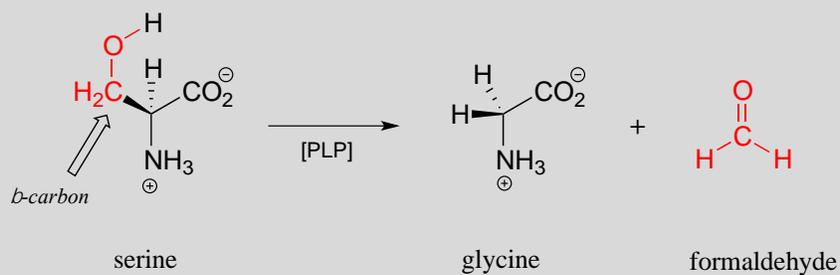
(It would be a good idea before reading this section to review aldol/retro-aldol and Claisen/retro-Claisen reaction mechanisms in sections 12.3 and 13.3, respectively)

So far we have seen PLP playing a role in breaking the bond between the α -carbon and its α -proton (in the racemization reaction), and the bond between the α -carbon and carboxylate carbon (in the decarboxylation reaction).

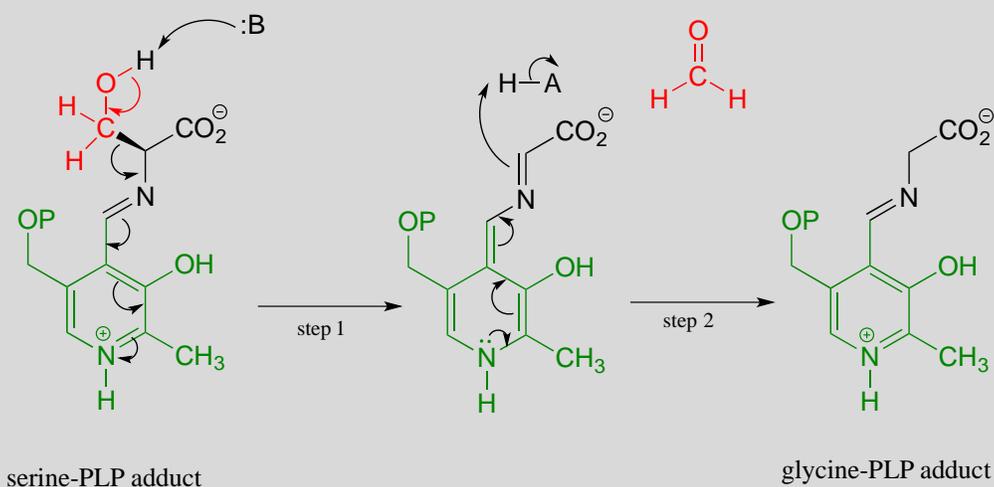


Other PLP-dependent enzymes catalyze cleavage of the bond between the α -carbon and the first carbon on the amino acid side chain, otherwise known as the β -carbon. In the serine degradation pathway, serine is first converted to glycine by a retro-aldol cleavage reaction. Although a reasonable mechanism could be proposed without the participation of PLP, this reaction in fact requires the coenzyme to assist in stabilization of the negative charge on the carbanion intermediate.

A PLP-dependent retro-aldol cleavage reaction
(serine hydroxymethyl transferase, EC 2.1.2.1)



Mechanism:

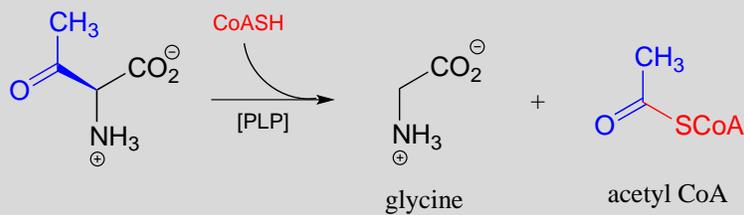


Note that, in this reaction just as in the racemase reaction described previously, the key intermediate is a PLP-stabilized carbanion, or quinonoid.

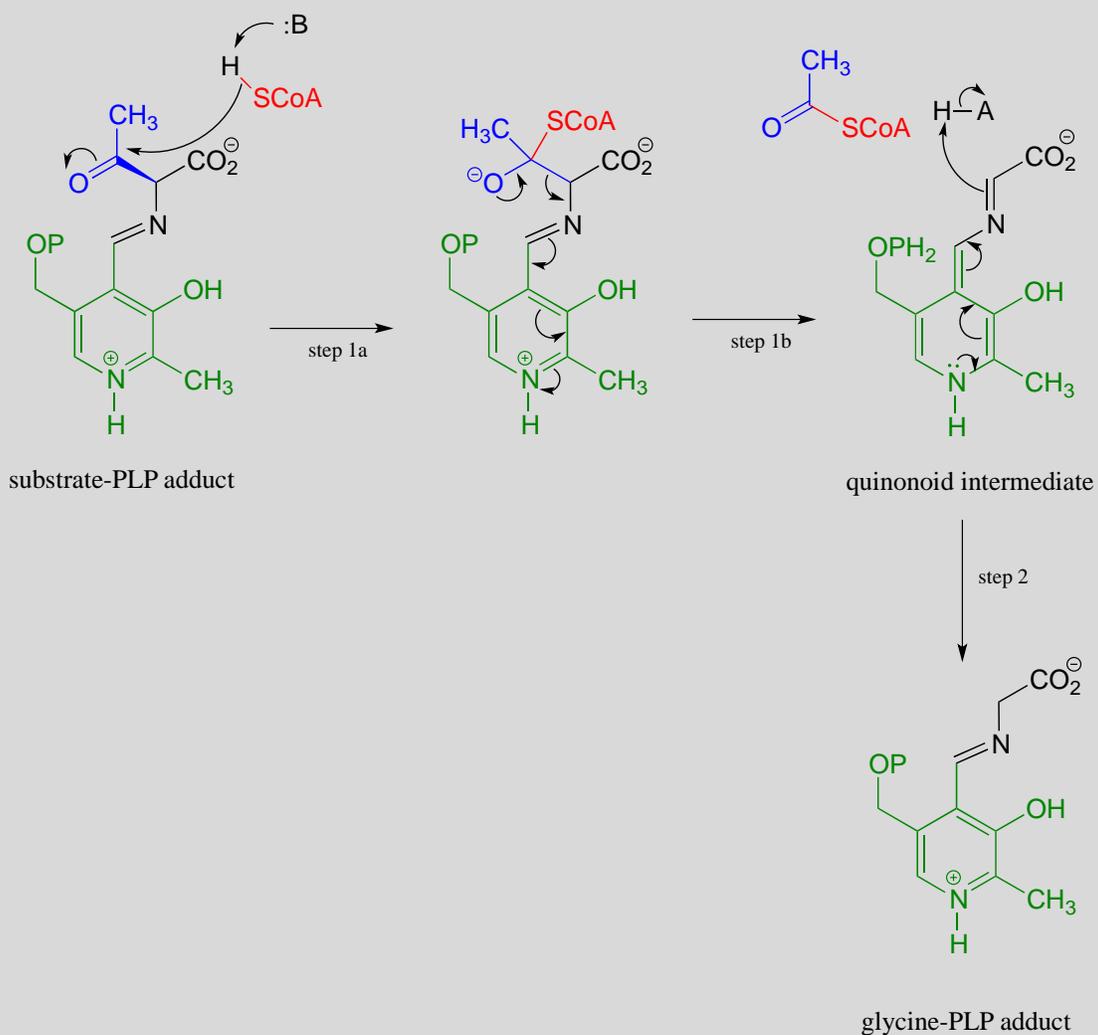
What happens to the (toxic!) formaldehyde produced in this reaction? We will see later in this chapter how the serine hydroxymethyltransferase enzyme goes on to use another coenzyme called tetrahydrofolate to prevent the formaldehyde from leaving the active site and causing damage to the cell.

PLP also assists in retro-Claisen cleavage reactions (section 13.3C), such as this step in the degradation of threonine. (EC 2.3.1.29)

A PLP-dependent retro-Claisen reaction:



Mechanism:

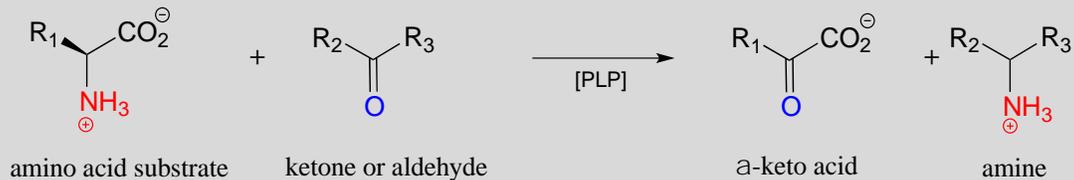


Notice how, like the retro-aldol reaction, the bond between the α -carbon and the β -carbon of the amino acid substrate is broken (in step 1b).

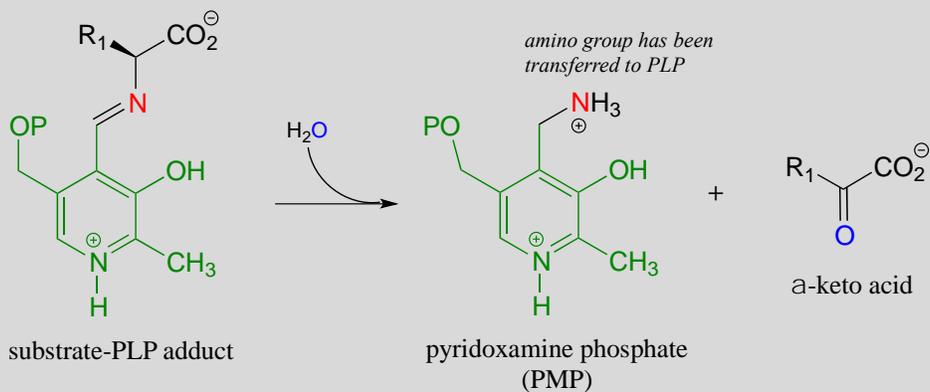
17.1-E: PLP-dependent transamination

One of the most important reaction types in amino acid metabolism is **transamination**, in which an amino group on a donor molecule (often an amino acid) is transferred to a ketone or aldehyde acceptor molecule.

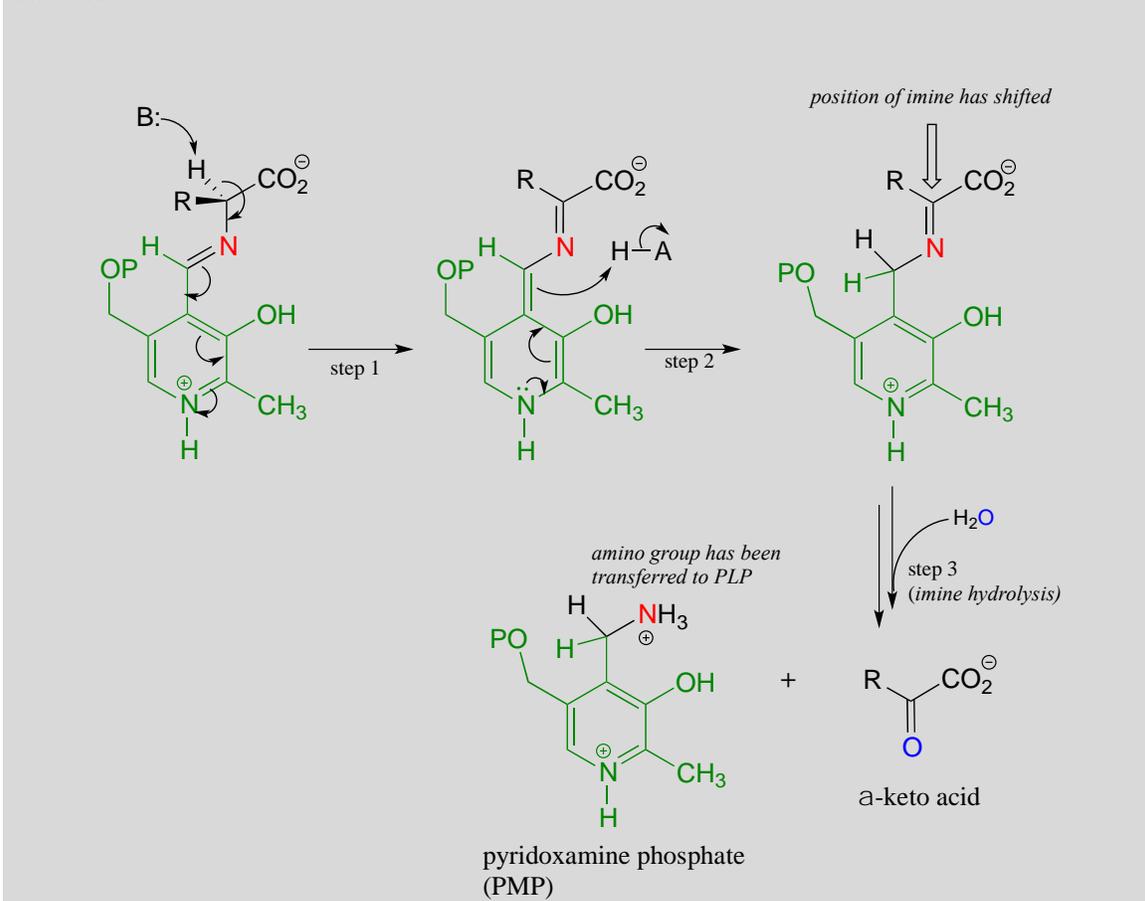
A transamination reaction:



Transamination phase 1 (transfer of amino group from amino acid substrate to coenzyme)



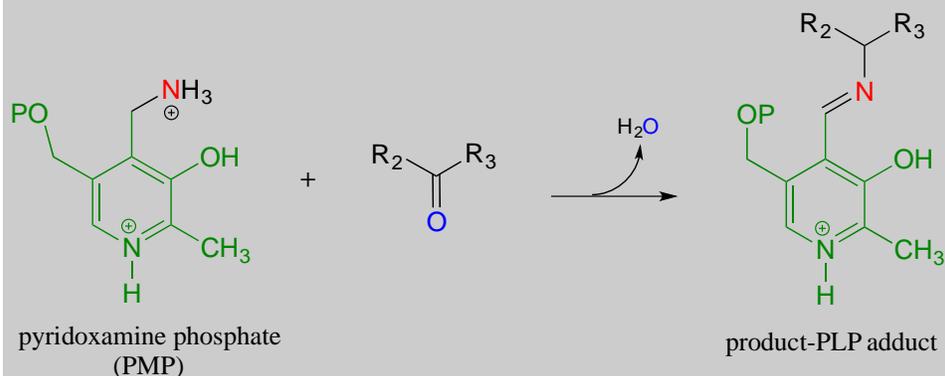
Mechanism:



Once again, step 1 is abstraction of the α -proton from the PLP-substrate adduct. However, in a transaminase reaction this initial deprotonation step is immediately followed by reprotonation at what was originally the aldehyde carbon of PLP (step 2 above), which results in a new carbon-nitrogen double bond (in other words, an imine) between the α -carbon and the nitrogen atom of the original amino acid. The repositioned imine group is then hydrolyzed (step 3 above), breaking the carbon-nitrogen bond, transferring the amino group to the coenzyme, and releasing an α -keto acid.

The coenzyme, which now carries an amine group and is called **pyridoxamine phosphate (PMP)**, next transfers the amine group to α -ketoglutarate (to form glutamate) through a reversal of the whole process depicted above.

Transamination reaction, phase 2
(transfer of amino group from coenzyme to acceptor molecule)

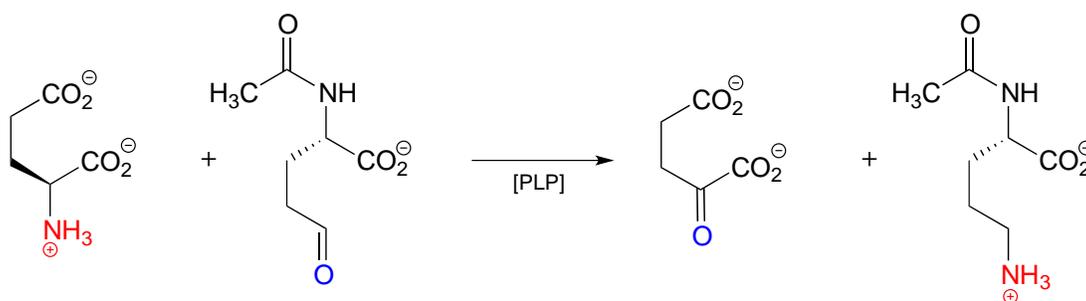


Mechanism:
see exercise below

In a transamination reaction, the PLP coenzyme not only provides an electron sink, it also serves as a temporary 'parking place' for an amino group as it is transferred from donor to acceptor.

Exercise 17.2: Show a complete, step-by-step mechanism for 'phase 2' of the transamination reaction above.

Here is an example of a transamination reaction in the arginine biosynthesis pathway: EC 2.6.1.11

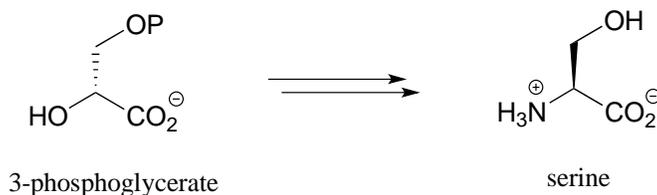


Exercise 17.3:

a) Draw arrows for the first mechanistic step of 'phase 2' of the above transaminase reaction.

b) Which carbon on the substrate side of the reaction will eventually become the α -carbon of arginine?

Exercise 17.4: Propose a pathway, with three enzymatic steps, for the biosynthesis of serine from 3-phosphoglycerate. Include a generalized ('-ase') enzyme name for each step. Glutamate plays a role in the process as an amino group donor.



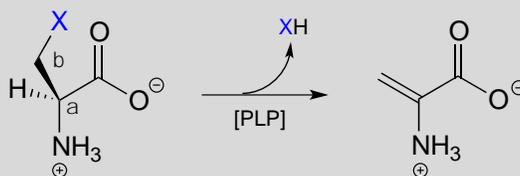
17.1F: PLP-dependent β -elimination and β -substitution

(Before starting this section, it would be a good idea to review E1cb β -elimination and conjugate addition reaction mechanisms in chapter 13.4)

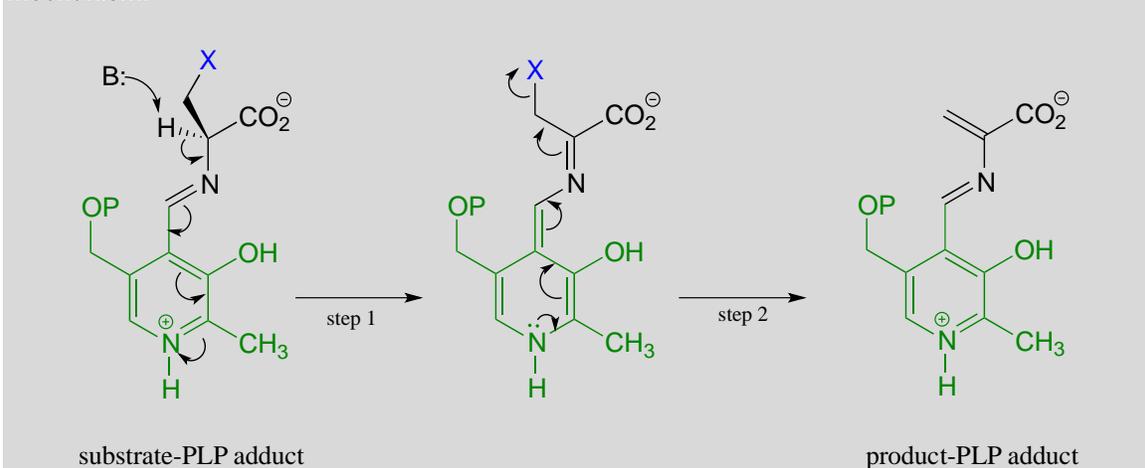
By now it should be pretty apparent that PLP is a pretty versatile coenzyme! Two more reaction types in the PLP toolbox are β -elimination and β -substitution on amino acid substrates.

In a PLP-dependent β -elimination reaction, the coenzyme simply helps to stabilize the carbanion intermediate of the E1cb mechanism:

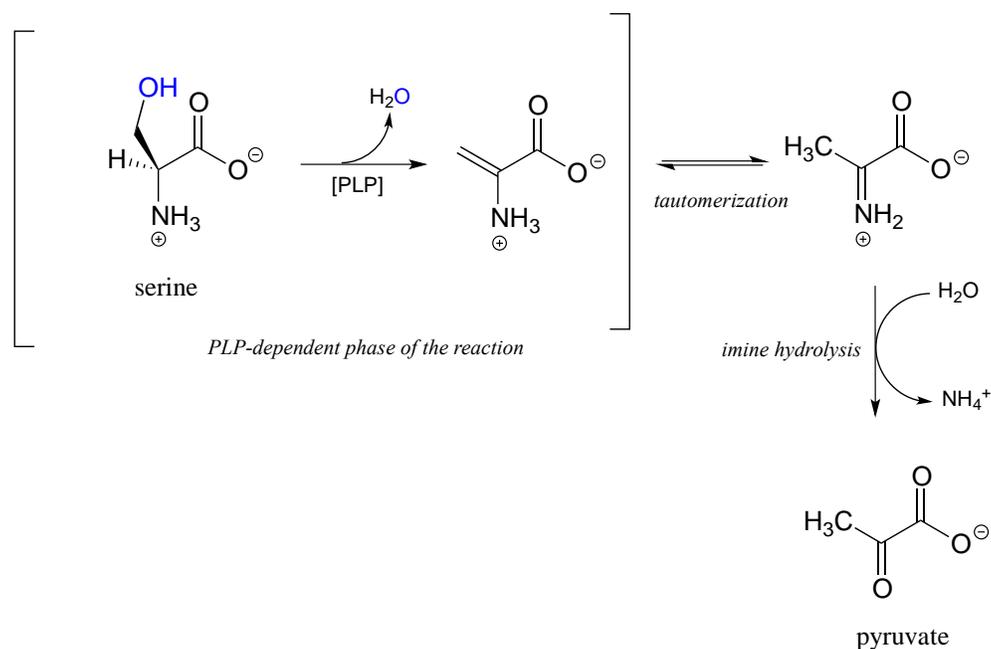
A PLP-dependent β -elimination reaction



Mechanism:

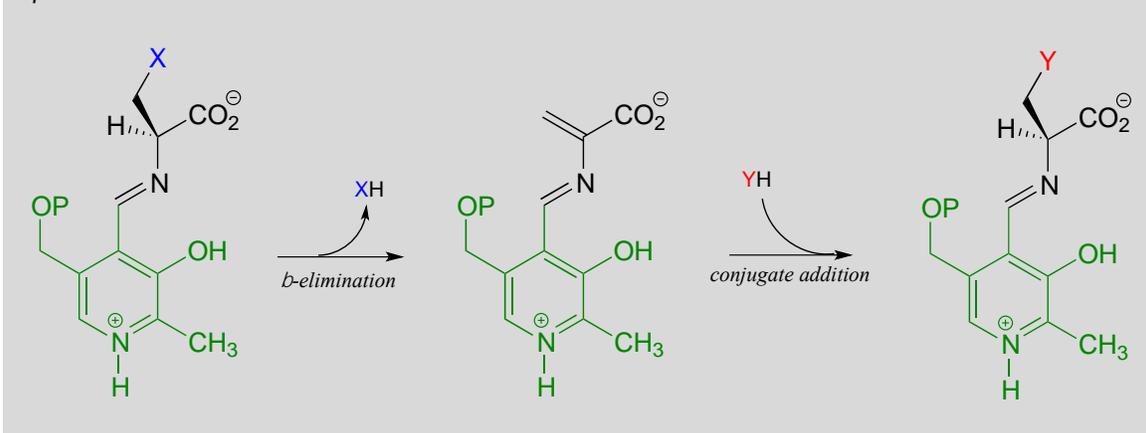


Serine dehydratase (EC 4.2.1.13) catalyzes a PLP-dependent β-elimination in the first step of the serine degradation pathway:

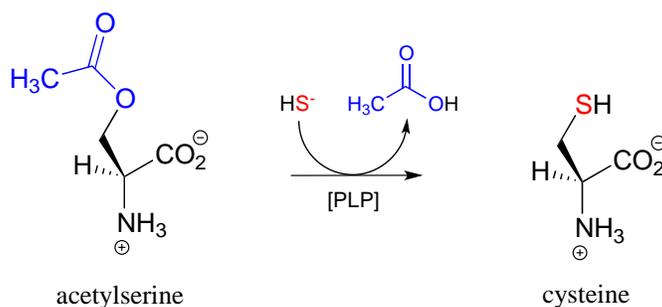


A β-substitution reaction is simply E1cb elimination followed directly by the reverse reaction (conjugate addition) with a different nucleophile (Y in the figure below):

A β -substitution reaction:



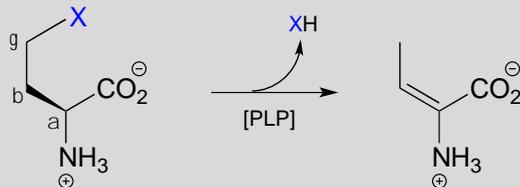
In many bacteria, the synthesis of cysteine from serine includes a PLP-dependent β -substitution step (EC 2.5.1.47).



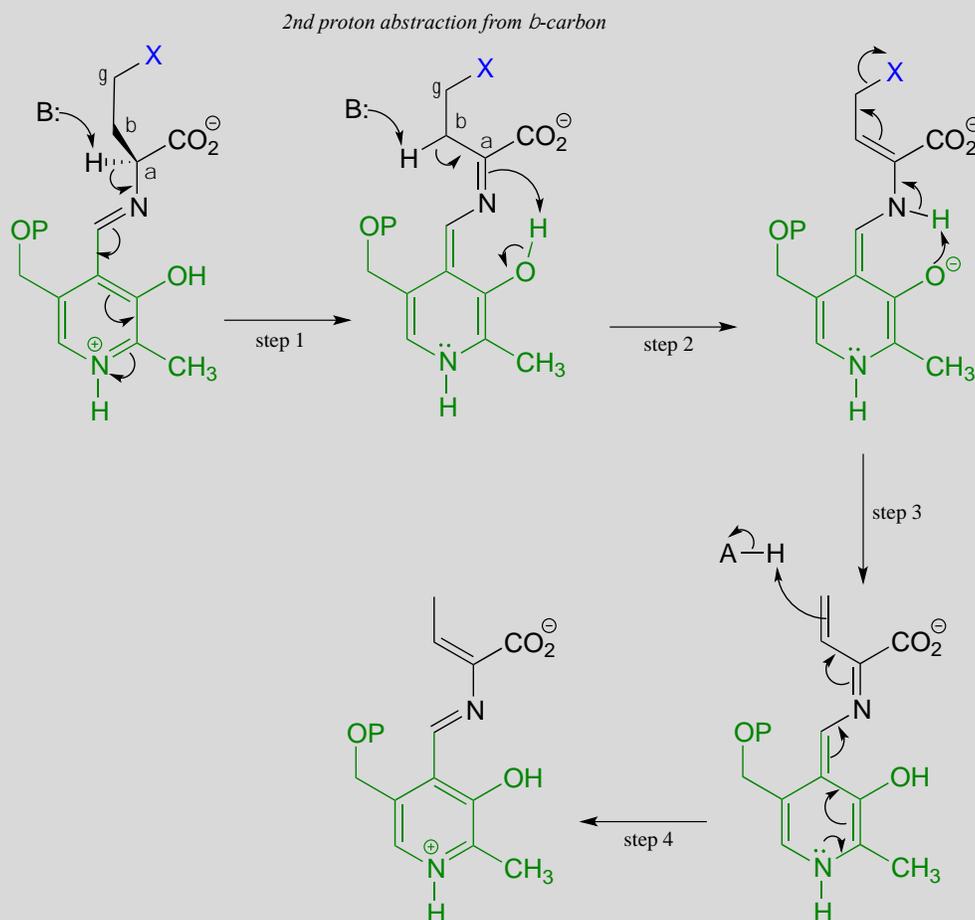
Exercise 17.5: Draw a mechanism for the conjugate addition phase of the reaction above (end with the cysteine-PLP adduct).

17.1G: PLP-dependent γ -elimination and γ -substitution reactions

The electron sink capability of PLP allows some enzymes to catalyze eliminations at the γ -carbon of some amino acid side chains, rather than at the β -carbon. The secret to understanding the mechanism of a γ -elimination is that PLP essentially acts as an electron sink *twice* - it absorbs the excess electron density from not one but two proton abstractions.

PLP-dependent γ -elimination

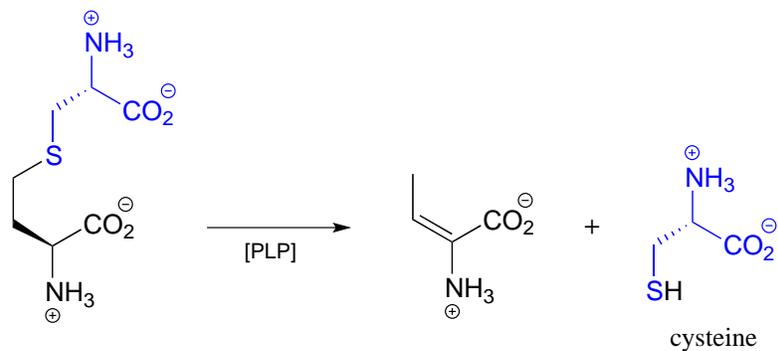
Mechanism:



In a familiar first step, the α -proton of the amino acid is abstracted by an enzymatic base, and the electron density is absorbed by PLP. Next comes the new part - before anything happens to the electron density from the first proton abstraction, a second proton, this time from the β -carbon on the side chain, is abstracted, forming an enamine intermediate (step 2). The phenolic proton on the pyridoxal ring of PLP donates a proton to the nitrogen. In step 3, the leaving group is expelled and a new π -bond forms between the β and γ carbons (step 3). This π -bond is short-lived, however, as the electron density from the first proton abstraction, which has been 'stored' in PLP all this time, flows back up to

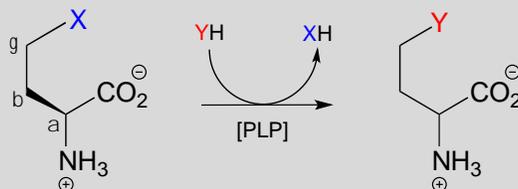
protonate the α -carbon (step 4), leaving the γ -elimination product linked to PLP via the usual imine connection.

An example is the cystathionine γ -lyase reaction in the methionine degradation pathway (EC 4.4.1.1):

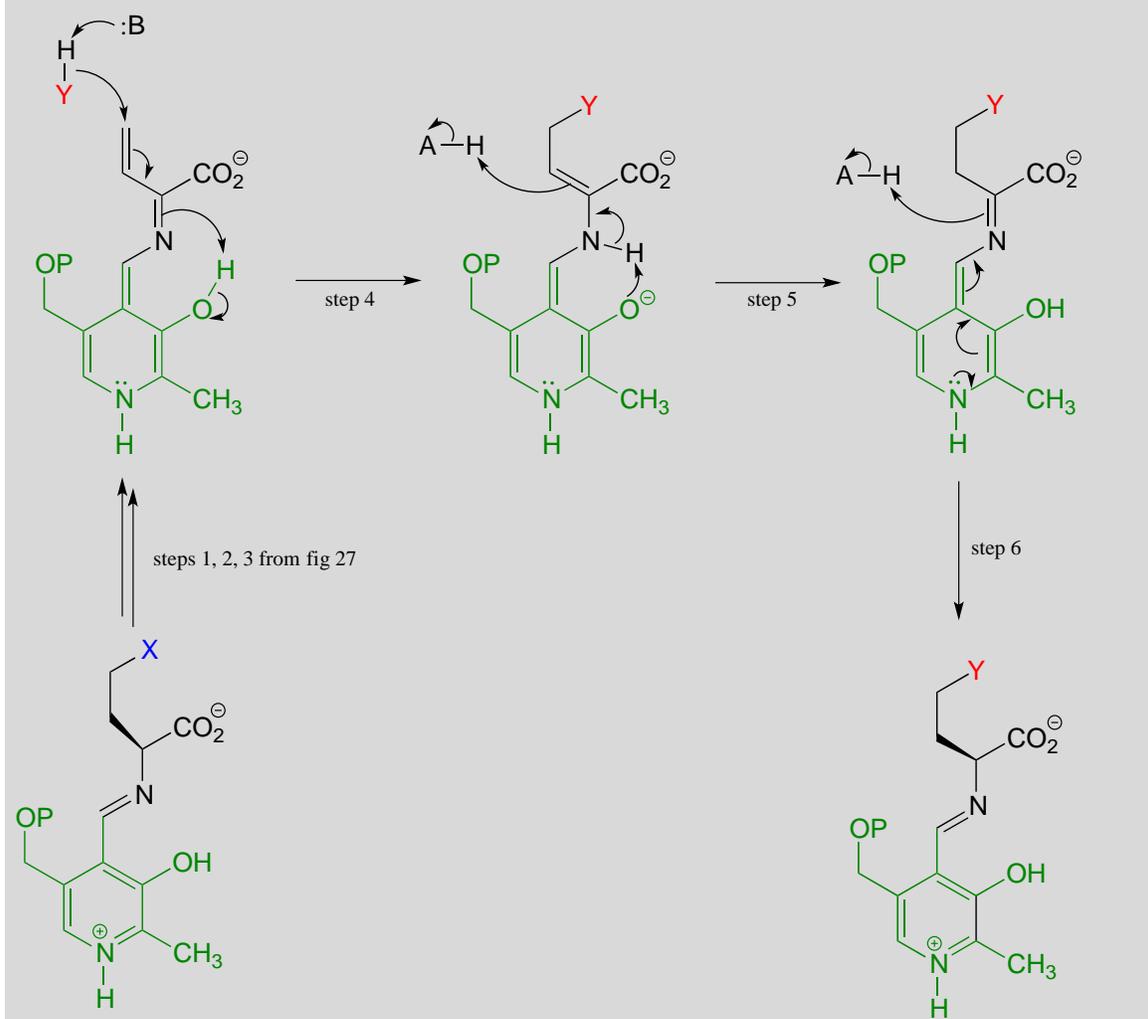


A related reaction is PLP-dependent γ -substitution, which again is simply γ -elimination of a leaving group (X in the figure below) followed directly by the reverse process (a γ -addition) with a different nucleophile ('Nu' in the figure below).

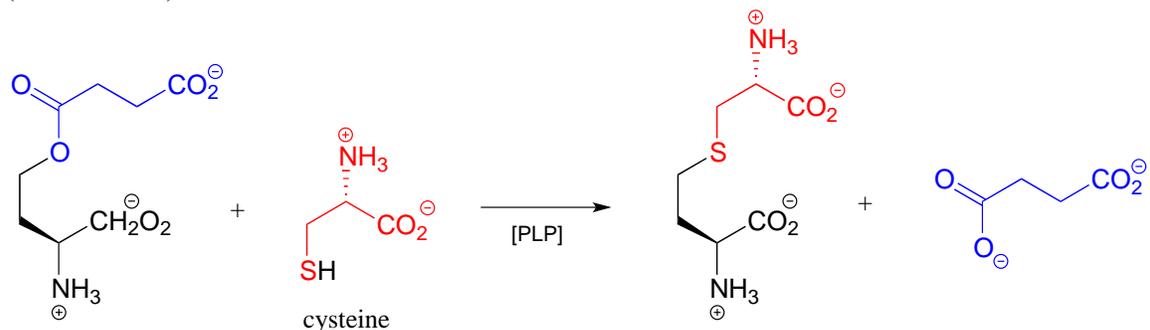
PLP-dependent γ -substitution:



Mechanism:



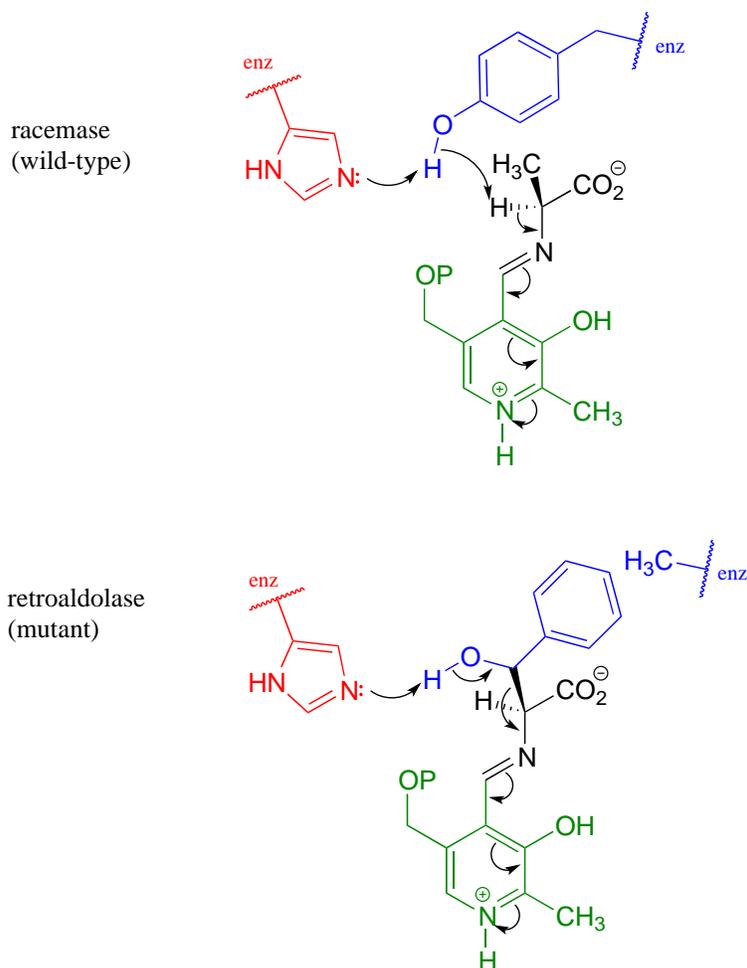
Below is a PLP-dependent γ -substitution reaction in the methionine degradation pathway (EC 4.2.1.22):



17.1H: Racemase to aldolase: altering the course of a PLP reaction

We have seen how PLP-dependent enzymes catalyze a variety of reaction types - racemization, retroaldol/retro-Claisen cleavage, transamination, elimination, and substitution - which, despite their apparent diversity, are all characterized by formation of a critical carbanion intermediate which is stabilized by the 'electron sink' property of the PLP coenzyme. Given this common mechanistic feature, it would be reasonable to propose that the active site architecture of these enzymes might also be quite close. This idea was nicely illustrated by an experiment in which researchers found that changing a single active site amino acid of PLP-dependent alanine racemase was sufficient to turn it into a retro-aldolase (*J. Am. Chem. Soc.* **2003**, *125*, 10158).

In the 'wild-type' (natural) alanine racemase reaction, an active site histidine (red in figure below) deprotonates a neighboring tyrosine residue (blue), which in turn acts as the catalytic base abstracting the α -proton of the substrate. When researchers changed the tyrosine to an alanine (using a technique called 'site-directed mutagenesis'), and substituted β -hydroxytyrosine for the alanine substrate, the new 'mutant' enzyme catalyzed a retro-aldol reaction.



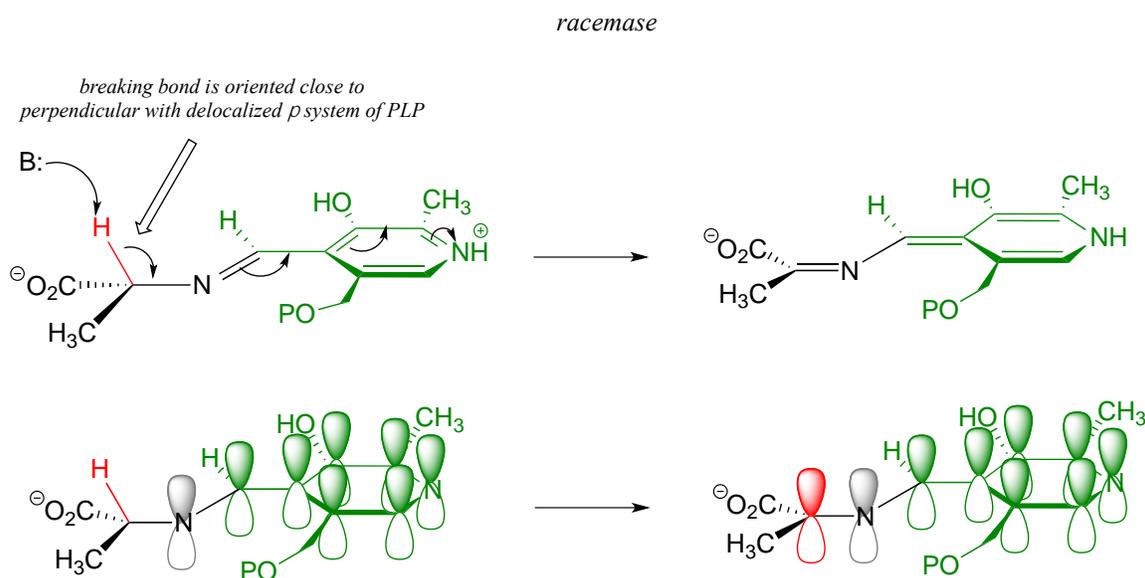
Notice what has happened here: the basic histidine, with no tyrosine to deprotonate because of the mutation, is instead positioned to abstract a proton from the β -hydroxyl group of the new substrate, setting up a retroaldol cleavage. That was all it took to change a racemase into a retroaldolase, because the necessary PLP electron sink system was all left in place. The researchers predicted correctly that the phenyl ring of β -hydroxy tyrosine would fit nicely in the space left empty due to the tyrosine to alanine change in the mutant enzyme's structure

These results underline the close mechanistic relationship between two PLP-dependent reactions which, at first glance, appear to be quite different - and suggest that PLP-dependent racemases and aldolases may have evolved from a common 'ancestor' enzyme.

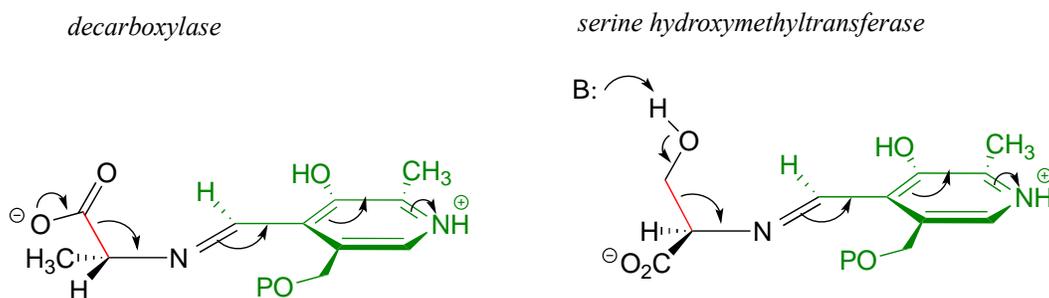
17.1I: Stereoelectronic considerations of PLP-dependent reactions

Recall that all PLP-dependent reactions involve the cleavage of one of the bonds coming from the α -carbon of an amino acid substrate, with the coenzyme serving as an 'electron sink' to stabilize the intermediate that results. PLP-dependent enzymes accelerate this bond-breaking step by binding the substrate-PLP adduct in a conformation such that the bond being broken is close to perpendicular to the plane formed by the conjugated π

system of PLP: this way, as the α -carbon transitions from sp^3 to sp^2 hybridization, the unhybridized p orbital is already oriented to overlap with the rest of the conjugated system. For example, in alanine racemase the first step is cleavage of the $C\alpha$ -H bond, so it must be that bond which is positioned near-perpendicular to the PLP plane:



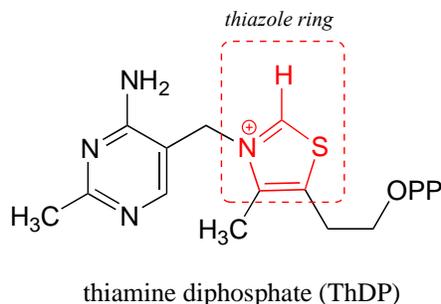
Likewise, in an amino acid decarboxylase, the $C\alpha$ -carboxylate bond is held near-perpendicular to the PLP plane, and in hydroxymethyltransferase, the $C\alpha$ - $C\beta$ bond is in the perpendicular orientation:



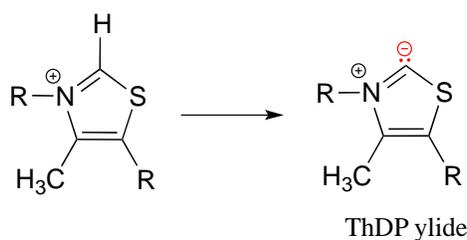
These are all good examples of how enzymatic catalysis is achieved, in part, by the ability of the active site to bind the substrate molecule in a specific conformation which contributes to the lowering of the activation energy of a key reaction step.

Section 17.2: Thiamine diphosphate (Vitamin B₁)

Thiamine diphosphate (ThDP, sometimes also abbreviated TPP or ThPP) is a coenzyme which, like PLP, acts as an electron sink to stabilize key carbanion intermediates. The most important part of the ThDP molecule from a catalytic standpoint is its thiazole ring.



The proton on the carbon between nitrogen and sulfur on the thiazole ring is weakly acidic, with a pKa of about 18.

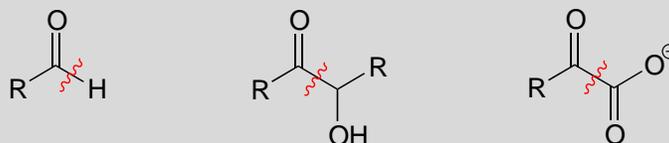


The reason for its acidity lies partly in the ability of the neighboring sulfur atom to accept, in its open *d*-orbitals, some of the excess electron density of the conjugate base. Another reason is that the positive charge on the nitrogen helps to stabilize the negative charge on the conjugate base. The deprotonated thiazole is called an **ylide**, which is a general term for a species with adjacent positively and negatively charged atoms.

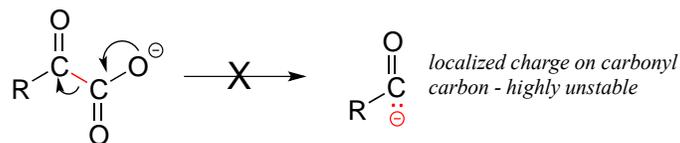
The negatively charged carbon on the ylide form of ThDP is nucleophilic, and as we shall soon see, the first step of most TPP-dependent reactions is nucleophilic attack of the ylide carbon on a carbonyl group of the reaction substrate.

ThDP plays a key role in a variety of reaction types, but the common theme in all ThDP-dependent reactions is cleavage of a bond adjacent to the carbonyl carbon of a ketone or aldehyde.

Thiamine diphosphate assists in breaking bonds next to a ketone or aldehyde:

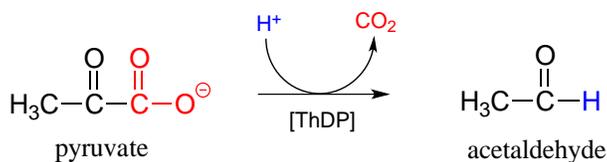


Consider this hypothetical decarboxylation step:



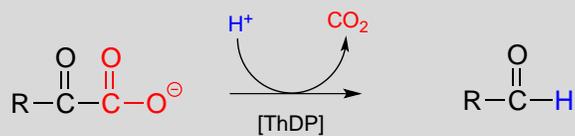
Hopefully, you can quickly recognize that this is *not* a chemically reasonable step, because the intermediate species which results from decarboxylation has a negative charge localized on the ketone carbon - a very unstable, unlikely intermediate indeed. (Recall from section 13.1 that decarboxylation steps usually result in intermediates in which the negative formal charge is delocalized to an oxygen or nitrogen - in other words, enolates or enamines.)

Now consider, however, a reaction going on in your cells right now, catalyzed by the enzyme pyruvate decarboxylase (EC 4.1.1.1):

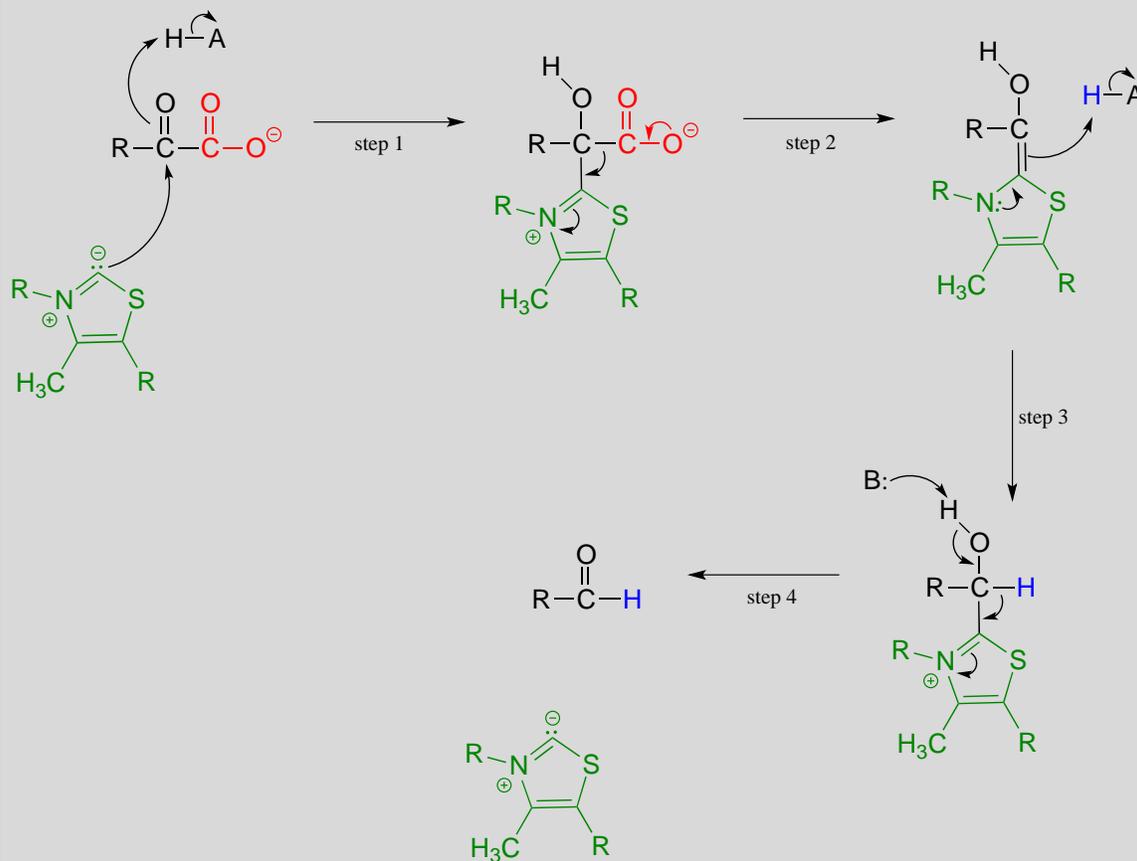


Somehow, the enzyme manages to accomplish this 'impossible' decarboxylation. How does this happen? Here is where the thiamine diphosphate coenzyme comes in.

A ThDP-dependent decarboxylation reaction (pyruvate decarboxylase):



Mechanism:



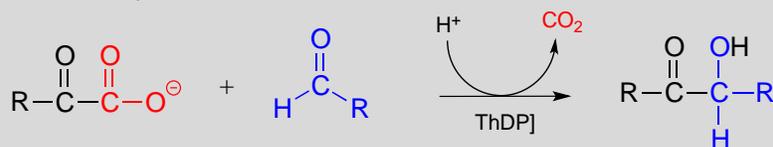
Upon binding to the enzyme's active site, ThDP quickly loses a proton. The nucleophilic ylide carbon then adds to the carbonyl carbon of pyruvate.

Look carefully at the intermediate that results from step 1 in the mechanism above. The thiazole ring of ThDP, once it has added to the carbonyl of pyruvate, provides an 'electron sink' to absorb the electrons from decarboxylation (step 2). Note which bond is breaking in step 2 - as was mentioned earlier, the common function of ThDP is to make possible the cleavage of a bond to a carbonyl carbon.

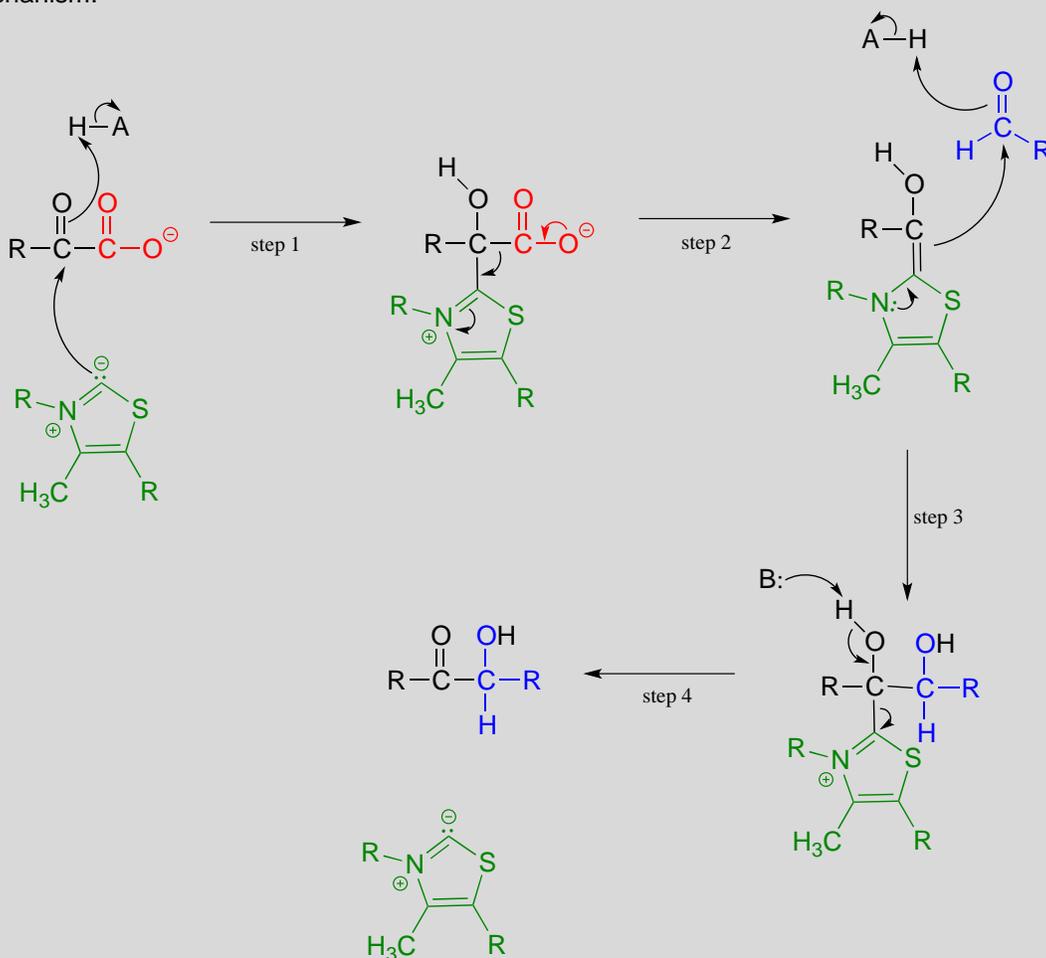
In step 3, the electrons from decarboxylation flow back to abstract a proton from an acidic group in the active site. All that remains is for the product to break free of thiamine in step 4.

Thiamine can also assist in decarboxylation-addition reactions:

ThDP-dependent decarboxylation-addition:

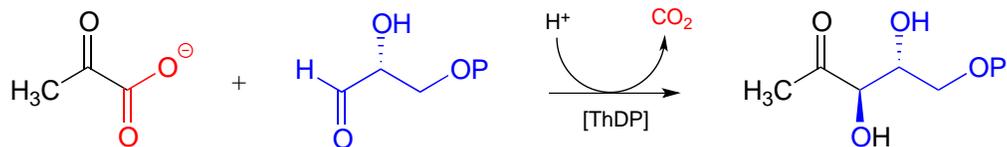


Mechanism:



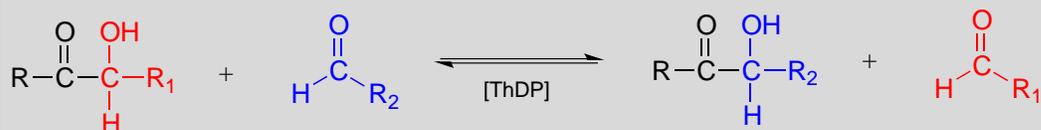
Here, the electron-rich intermediate formed from the decarboxylation step (step 2) simply goes on to act as a nucleophile rather than as a base, adding to the carbonyl group of an aldehyde or ketone (step 3). As before, the product breaks free of ThDP in step 4.

An example is the first step in the biosynthetic pathway for isoprenoid compounds in bacteria:

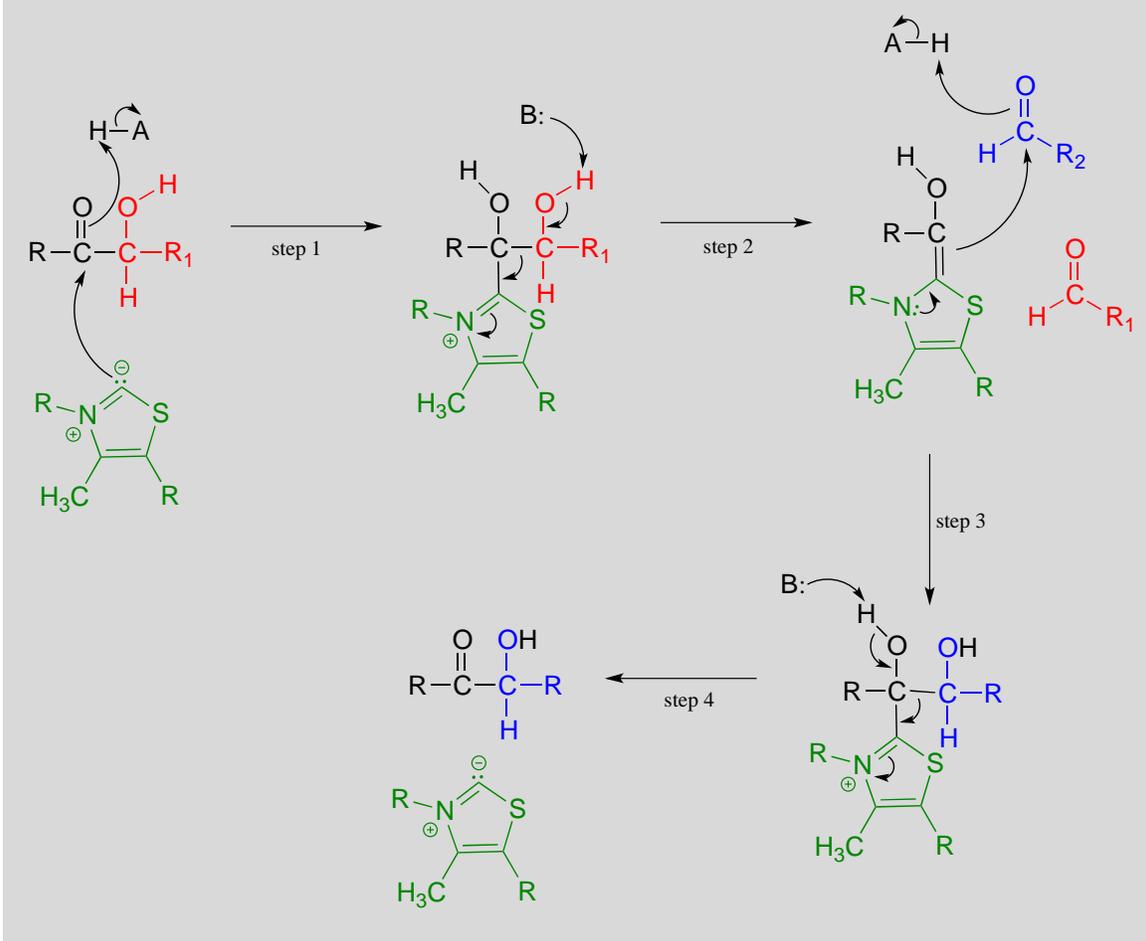


Transketolase, a ThDP-dependent enzyme in the pentose phosphate pathway of sugar metabolism, catalyzes a carbon-carbon bond break step, followed by a carbon-carbon bond forming step. The substrates and products are at similar energy levels, so the reaction is completely reversible.

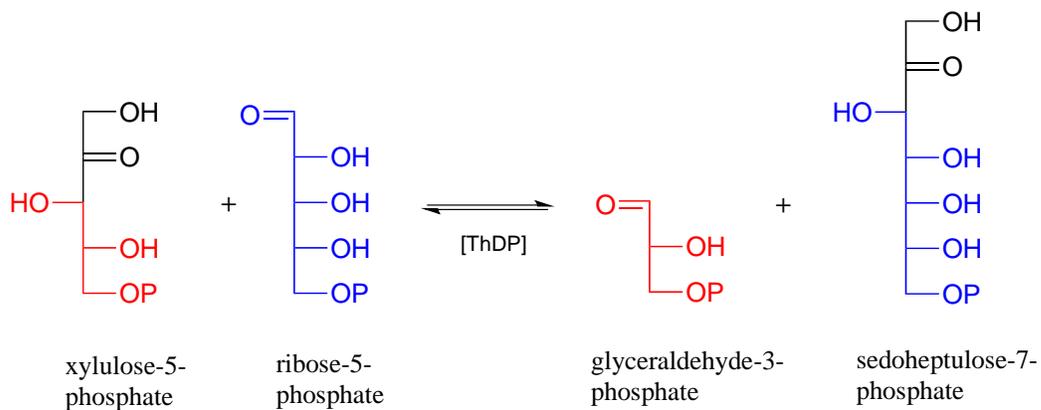
Transketolase reaction:



Mechanism:

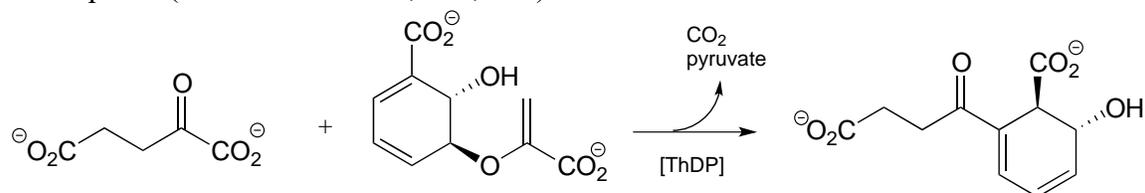


Below is an actual example of a transketolase-catalyzed transformation from the pentose phosphate pathway (shown in Fischer projections, as is common for sugar structures).

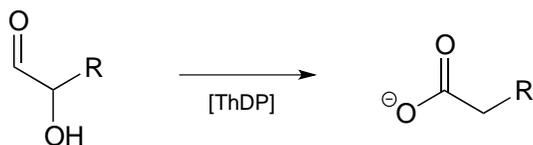


Exercise 17.6: As was mentioned above, the transketolase reaction is highly reversible. Do you think the same can be said for the decarboxylation and decarboxylation-addition reactions we saw in this section? Why or why not?

Exercise 17.7: (Challenging!) Propose a mechanism for the reaction below. Hint: This is a ThDP-facilitated decarboxylation/ Michael addition, followed by E1cb elimination of pyruvate. A Michael addition is the name for a conjugate addition with a carbon nucleophile. (*J. Mol. Biol.* **2010**, 401, 253).



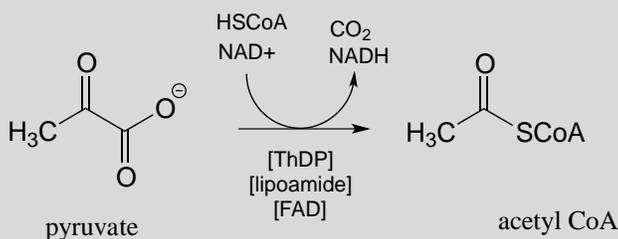
Exercise 17.8: Propose a mechanism for the reaction below. *Hint:* the mechanism can be described as a ThDP-facilitated dehydration step, followed by a tautomerization step, followed by a *hydrolytic* expulsion of ThDP (a different kind of ThDP expulsion from what we have seen so far!)



Section 17.3: Thiamine diphosphate, lipoamide and the pyruvate dehydrogenase reaction

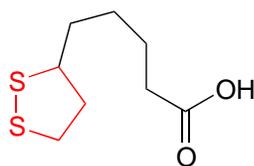
The enzyme pyruvate dehydrogenase is one of the most central of all the enzymes of central metabolism: by converting pyruvate to acetyl-CoA, it links glycolysis (where glucose is broken down into pyruvate) to the citric acid cycle, into which carbons enter in the form of acetyl-CoA. Five coenzymes are involved: coenzyme A, nicotinamide, thiamine diphosphate, FAD, and finally lipoamide, one which is new to us at this point.

Reaction catalyzed by pyruvate dehydrogenase:

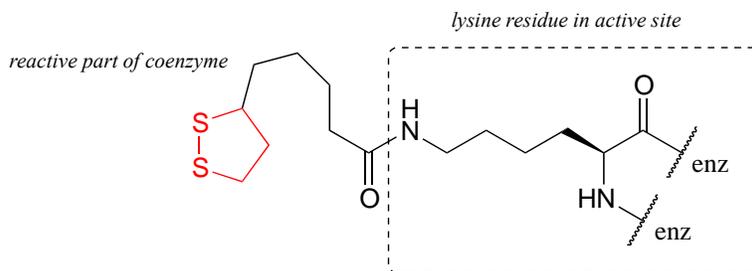


You will learn more about the structure and metabolic role of this complex and remarkable enzyme in a biochemistry course. Here, we will focus on the multi-step organic reaction it catalyzes, which we are at long last equipped to understand.

Looking at the reaction, you should recognize that, first of all, the pyruvate substrate is being oxidized - it starts out as a ketone, and ends up as a thioester, losing carbon dioxide in the process. Ultimately, the oxidizing agent in this reaction is NAD^+ , but the reduction of NAD^+ is linked to the oxidative decarboxylation of pyruvate by FAD and a disulfide-containing coenzyme called **lipoamide**, which is lipoic acid attached by an amide linkage to a lysine residue on the enzyme.



lipoic acid

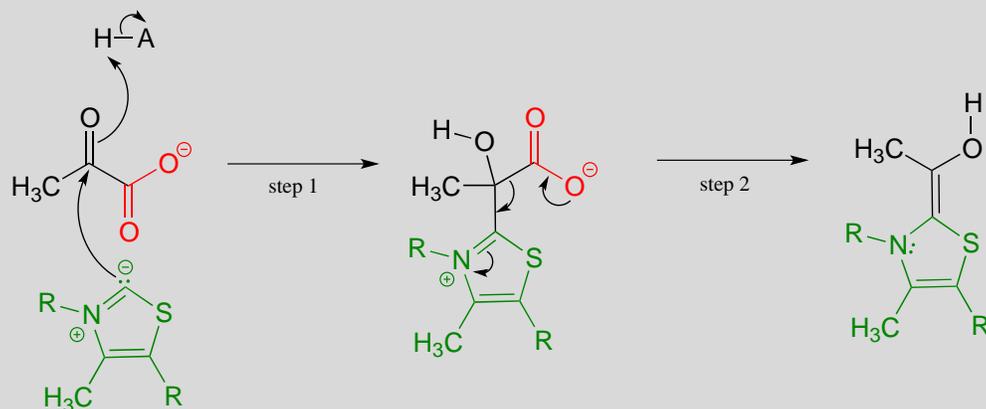


lipoamide
(linked to lysine residue in enzyme)

The second thing to notice is that, because the reaction involves breaking the bond between the ketone carbon and an adjacent carbon, thiamine diphosphate (ThDP) coenzyme is required. In fact, the first phase of the reaction (steps 1 and 2 below) is identical to that of pyruvate *decarboxylase*, an enzyme we discussed a few pages ago.

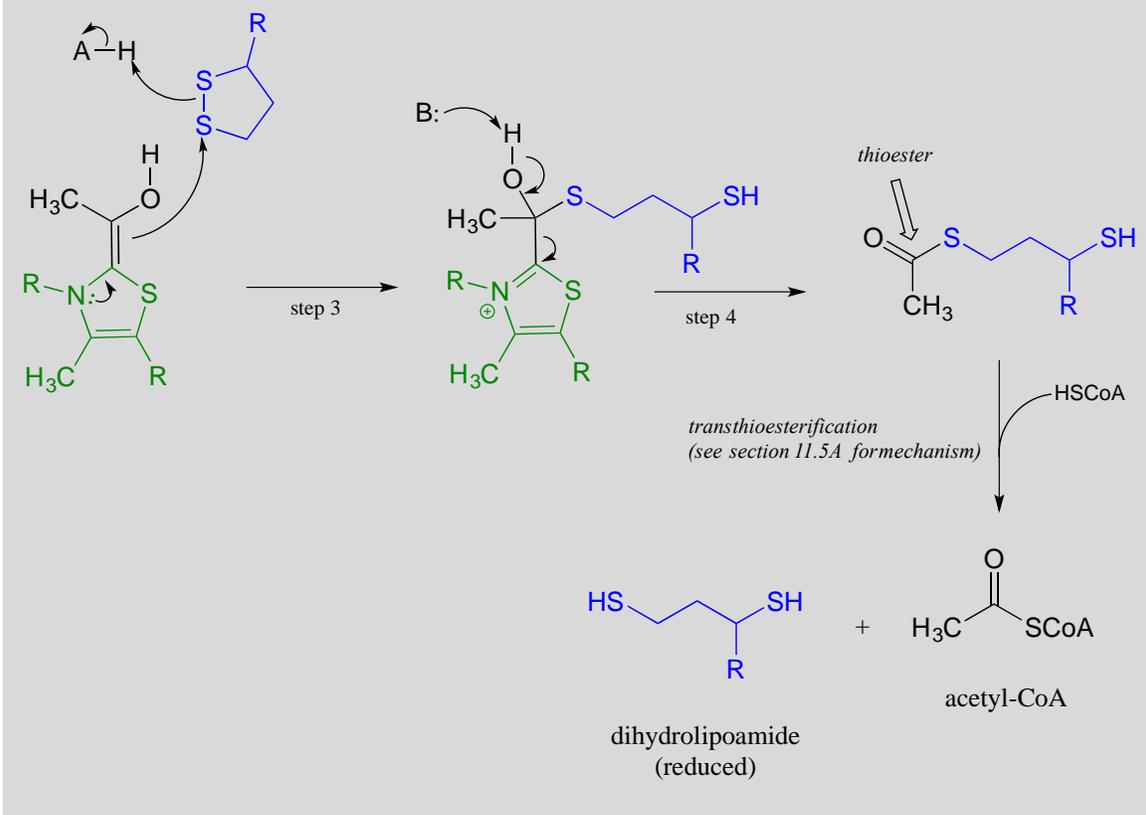
The pyruvate decarboxylase reaction mechanism

Phase 1: Decarboxylation of pyruvate



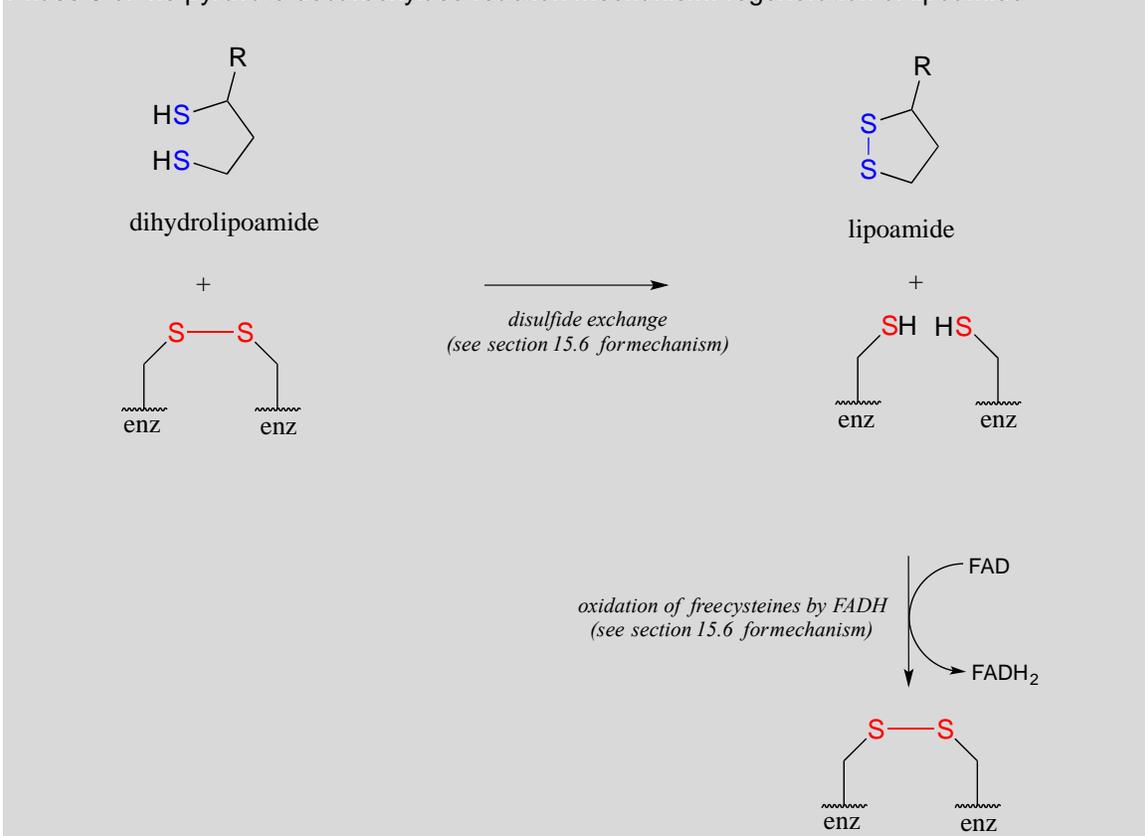
The ThDP-stabilized carbanion then acts as a nucleophile, cleaving the disulfide bridge of lipoamide (step 3 below). It is in this step that oxidation of the substrate is actually occurring. After the resulting thioester product is released from ThDP (step 4 below), it undergoes transesterification form acetyl-CoA, the product of the reaction.

Phase 2 of the pyruvate decarboxylase reaction mechanism: lipoamide-mediate oxidation to acetyl-CoA



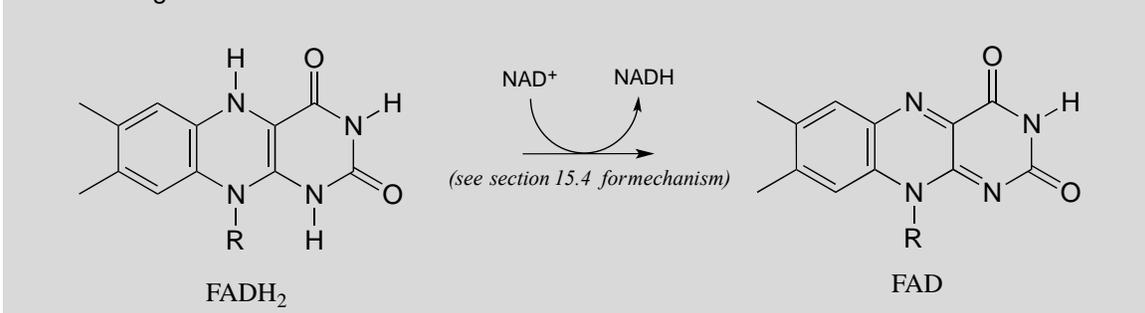
We are not done yet! In order for the catalytic cycle to be complete, the reduced dihydrolipoamide must be regenerated back to its oxidized state through disulfide exchange with a disulfide bond on the enzyme. The pair of enzymatic cysteines is then oxidized back to disulfide form by an FAD-dependent reaction.

Phase 3 of the pyruvate decarboxylase reaction mechanism: regeneration of lipoamide



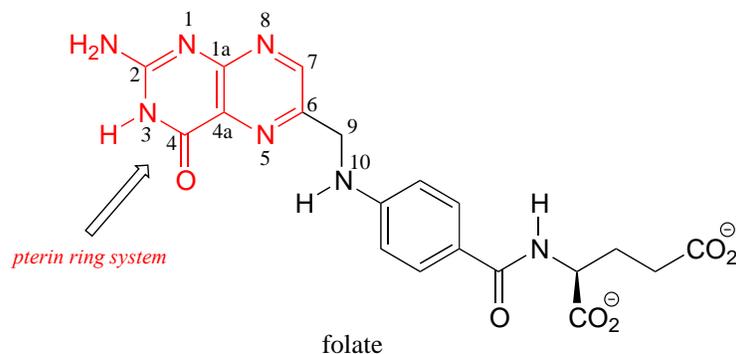
Finally, FAD is regenerated with concurrent reduction of NAD⁺:

Phase 4: Regeneration of FADH₂:



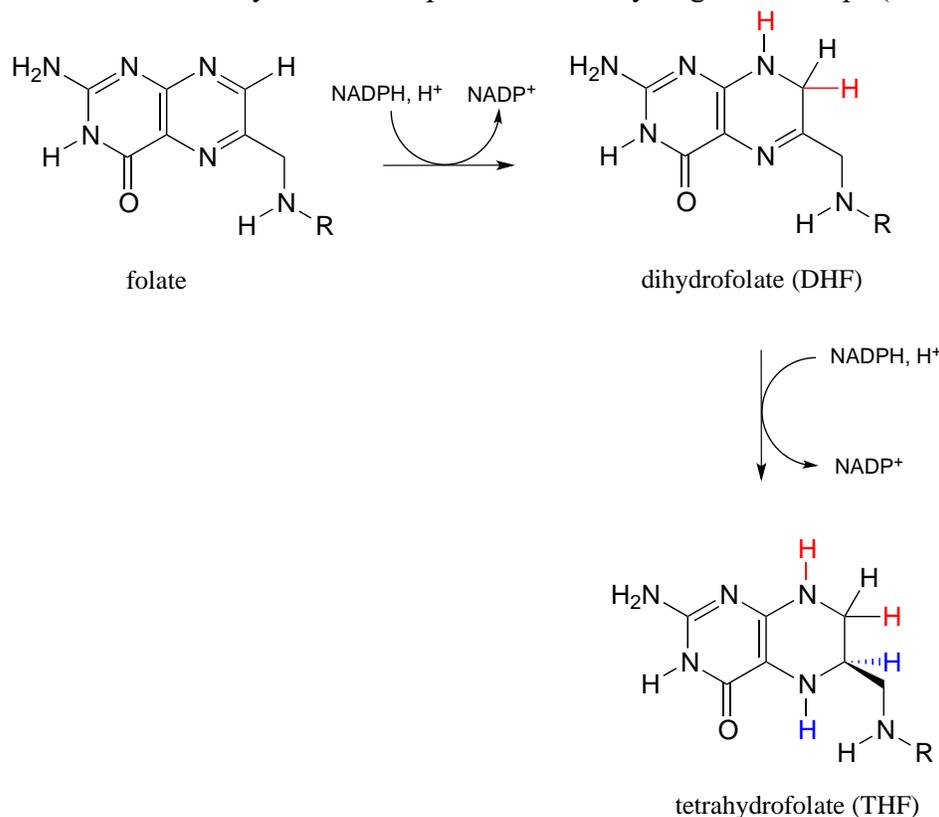
Section 17.4: Folate

Folate, or vitamin B₉, is essential for a variety of important reactions in nucleotide and amino acid metabolism. The reactive part of folate is the **pterin** ring system, shown in red below. The conventional atom numbering system for folate is also indicated.

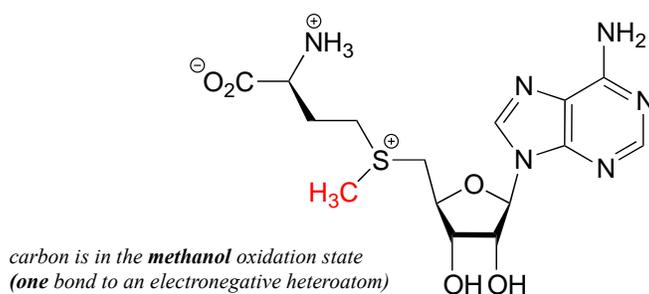


17.4A: Active forms of folate

Folate is active as a coenzyme in its reduced forms, dihydrofolate and tetrahydrofolate, which are formed by NADPH-dependent imine hydrogenation steps (section 15.3).

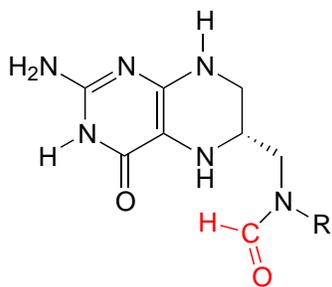


The metabolic role of folate is to serve as a donor or acceptor in single-carbon transfer reactions. How is folate different from *S*-adenosylmethionine (SAM, section 8.8A) which also serves as a single-carbon donor? Recall that SAM donates a single carbon in the form of a methyl group: essentially, the single carbon of SAM is in the methanol (CH₃OH) oxidation state, because it has only one bond to a heteroatom (specifically, to sulfur). (Refer to section 15.1 for a review of oxidation states).



S-adenosylmethionine
(SAM)

Folate coenzymes, on the other hand, can carry a single carbon in the *formaldehyde* and *formate* oxidation states, in addition to the methanol oxidation state. By 'formaldehyde' and 'formate' oxidation state, we mean that the carbon has two and three bonds to heteroatoms, respectively.

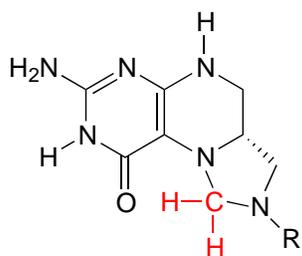


formyltetrahydrofolate
(f-THF)

carbon is in the **formate** oxidation state
(**three** bonds to electronegative heteroatoms)



formate

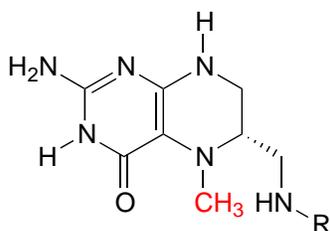


methylenetetrahydrofolate
(CH₂-THF)

carbon is in the **formaldehyde** oxidation state
(**two** bonds to electronegative heteroatoms)

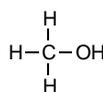


formaldehyde



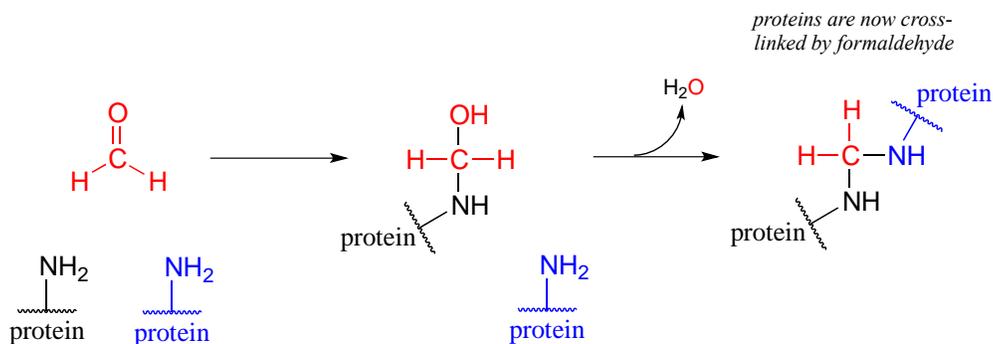
methyltetrahydrofolate
(CH₃-THF)

carbon is in the **methanol** oxidation state
(**one** bond to an electronegative heteroatom)



methanol

Some key reactions in nucleic acid and amino acid metabolic pathways involve transfer of a single carbon in the formaldehyde or formate states. However, this could present problems. Formaldehyde by itself is very toxic: in particular, it tends to spontaneously form unwanted crosslinks between amine groups (eg. lysine side chains) in proteins.

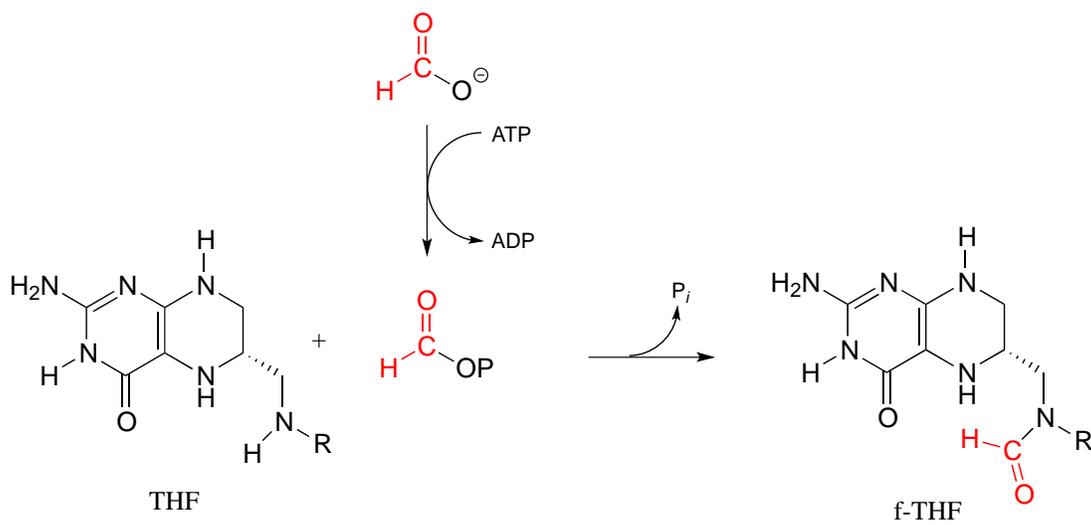


Free formaldehyde is *too reactive*, and would cause damage to a cell. The CH₂-THF coenzyme is stable in solution, but in the active site of certain enzymes it is reactive enough to serve as a formaldehyde donor, as we will see shortly.

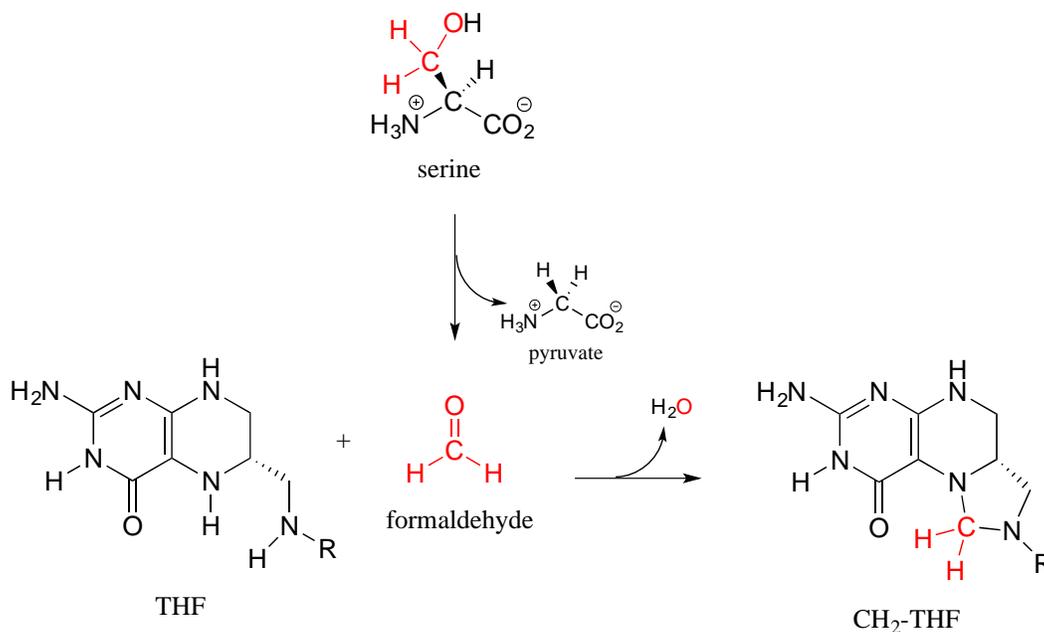
Free formate, on the other hand, is a carboxylate, and we know from chapter 11 that carboxylates are *not reactive* in acyl substitution steps. Formate could be activated by phosphorylation, of course, but the resulting formyl phosphate would be *too reactive* in many enzyme active sites. A 'happy medium' has been found in which carbons in the formate oxidation state are carried by folate in the form of f-THF: once again, the carbon donor is stable in solution, but sufficiently reactive in certain enzyme active sites to accomplish controlled transfer of a formate group.

17.4B: Formation of formyl-THF and methylene-THF

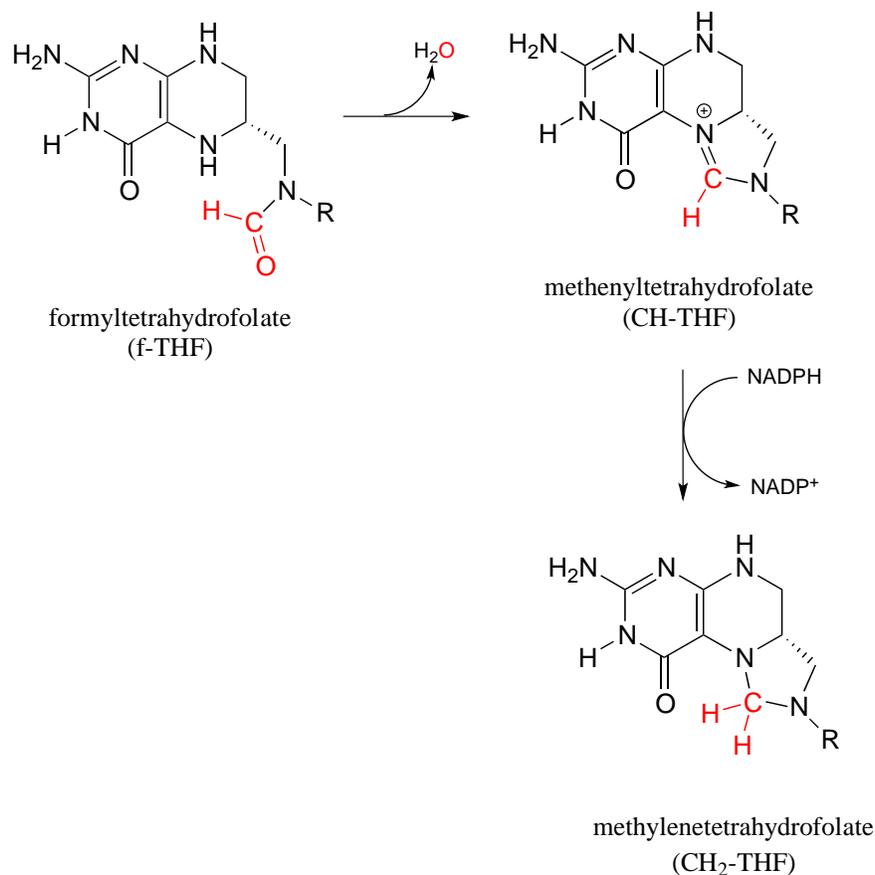
Formyltetrahydrofolate (f-THF) is formed from THF and a formate molecule which has been activated by phosphorylation (formyl phosphate, as stated in the paragraph above, is a high reactive intermediate, but is held inside the enzyme's active site for immediate reaction with the incoming amine group of THF).



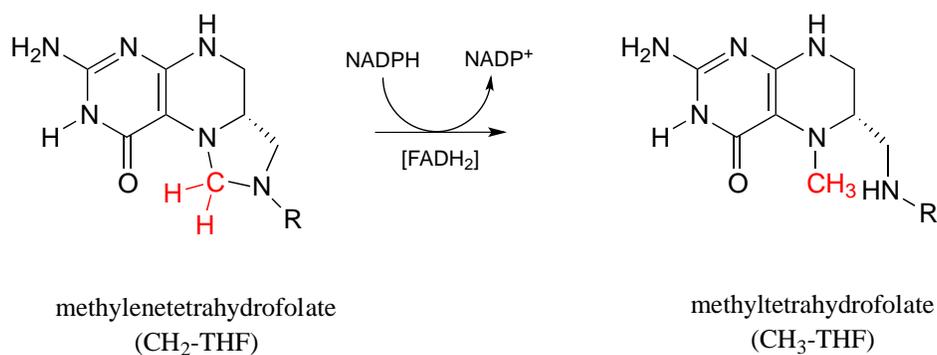
There are two main metabolic routes to CH₂-THF. One route is just the last step of the serine hydroxymethyltransferase reaction we have already seen in section 17.1D: the formaldehyde formed in the PLP-dependent phase of the reaction stays in the active site, and the oxygen is displaced by successive attacks from the amine nucleophiles at the 5 and 10 positions of THF. (Notice the similarity to the formaldehyde-protein crosslinking reaction shown earlier in this section.)



In the second route, f-THF is dehydrated, then the resulting methenyl-THF intermediate is reduced by NADPH.

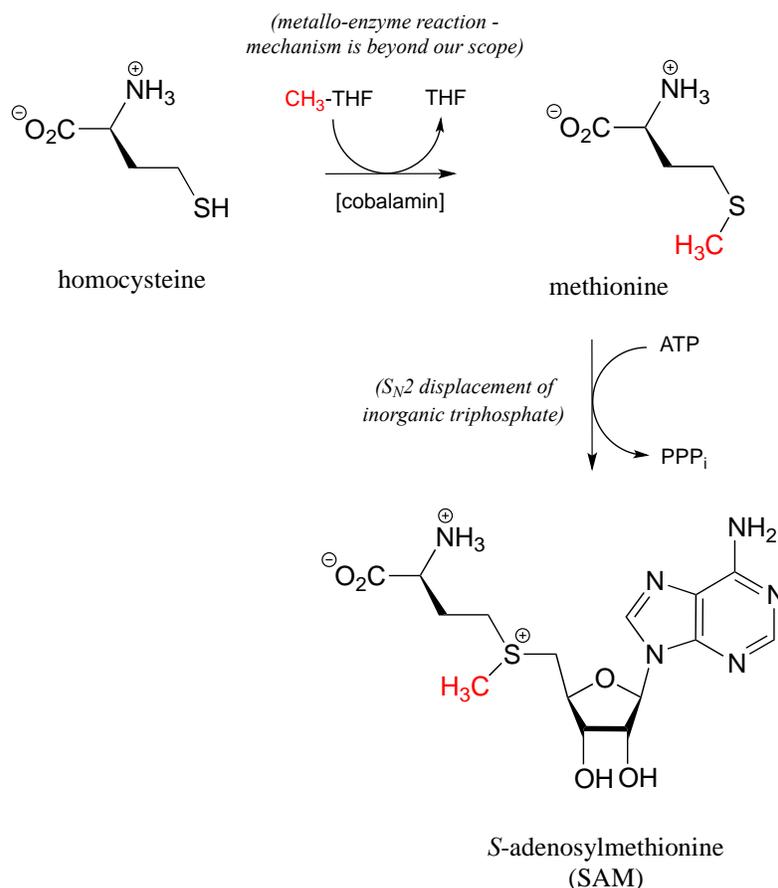


Methylene-THF (CH₂-THF) is reduced to methyl-THF (CH₃-THF) in a flavin-dependent reaction.



Recall from the introduction to this chapter that babies who were in the womb during the Dutch Hunger Winter faced a higher risk, when they reached adulthood, of conditions such as obesity and schizophrenia, most likely caused by disruptions in the *S*-adenosylmethionine-dependent methylation of their DNA, which was in turn caused by

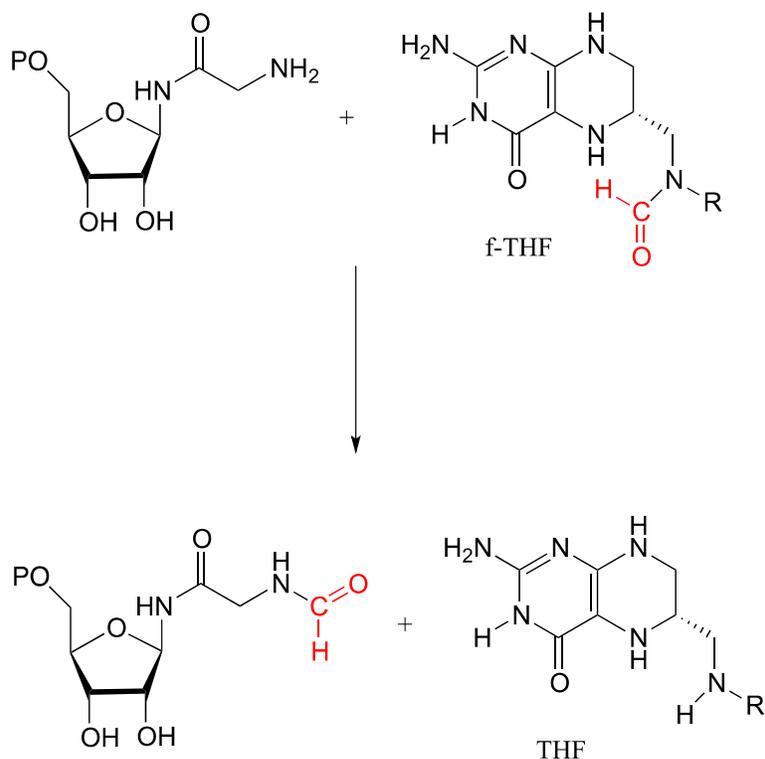
their mothers not getting enough folate. $\text{CH}_3\text{-THF}$ is the source of the methyl group in methionine, which ultimately becomes the methyl group in SAM. The methylation of homocysteine to methionine (below) involves a cobalt-containing coenzyme called cobalamin, but the mechanism for this reaction is beyond the scope of our discussion. The second reaction below (formation of SAM) is simply an $\text{S}_{\text{N}}2$ displacement of the inorganic triphosphate leaving group on ATP by the nucleophilic sulfur in methionine.



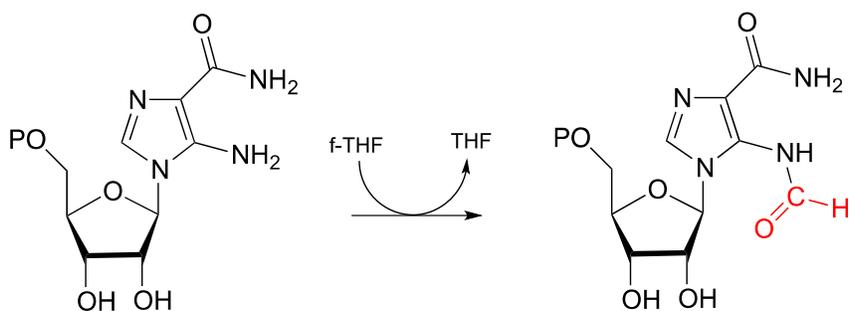
17.4C: Single-carbon transfer with formyl-THF

There are two important f-THF-dependent formylation steps in the biosynthetic pathways for purine nucleophiles. Both are simply transamidation reactions: in other words, conversion by the nucleophilic acyl substitution mechanism (chapter 11) of one amide to another.

Glycinamide ribonucleotide transformylase reaction:



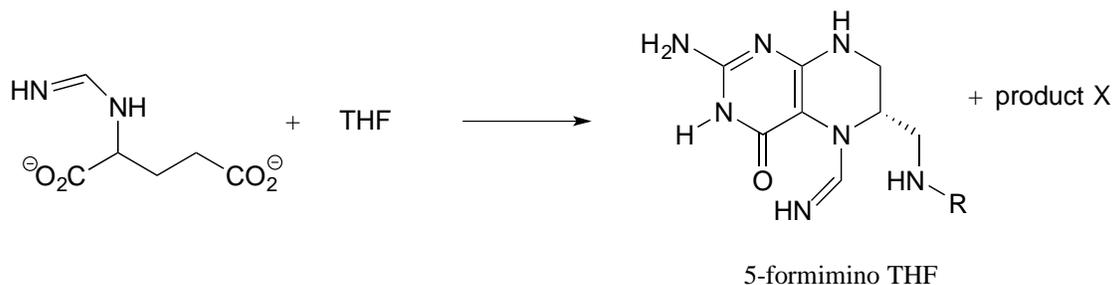
Aminoimidazole carboxamide transformylase reaction:



Formylation of amine groups plays an important role in our immune system. The N-terminal amino acid of bacterial proteins is typically an N-formylmethionine, a modified amino acid not found in eukaryotes. We have immune system receptor proteins that can recognize these formylated bacterial proteins, thus marking a bacterial pathogen for destruction.

Exercise 17.9: Which of the two transformylase reactions above would you expect to be more kinetically favorable? Explain your reasoning.

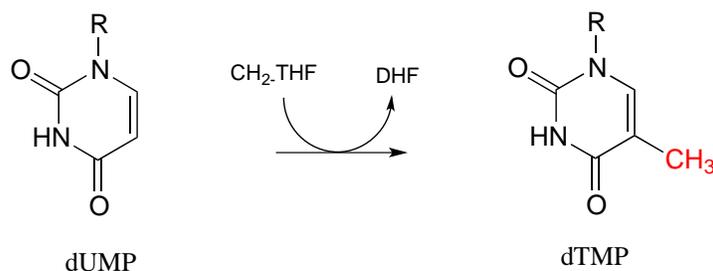
Exercise 17.10: Predict the structure of 'product X' in the reaction below, from the histidine degradation pathway.



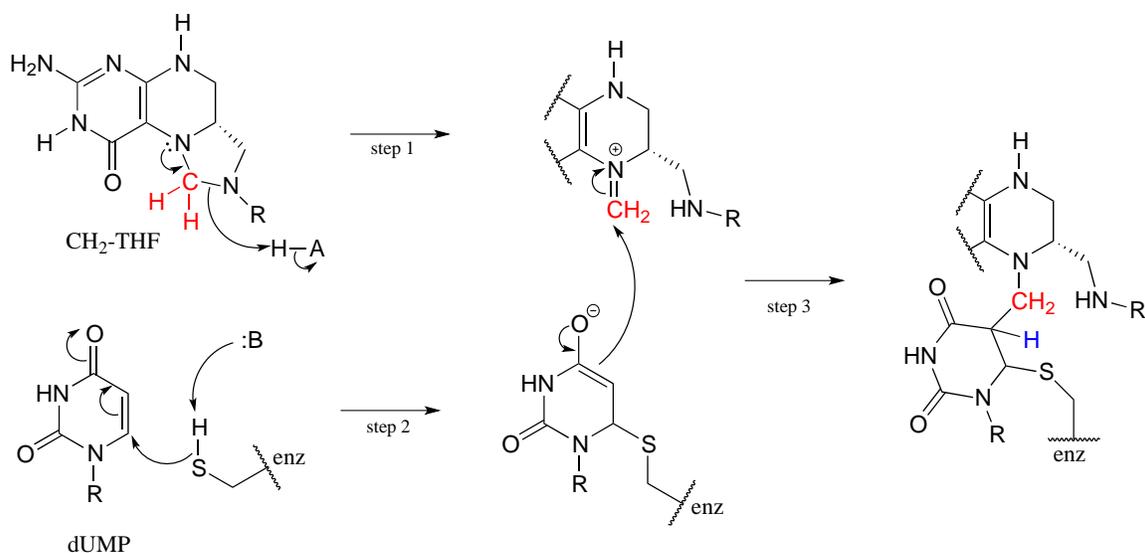
17.4D: Single-carbon transfer with methylene-THF

CH₂-THF serves as a single-carbon donor in a somewhat complicated reaction in the biosynthesis of the DNA monomer deoxythymidine monophosphate (dTMP).

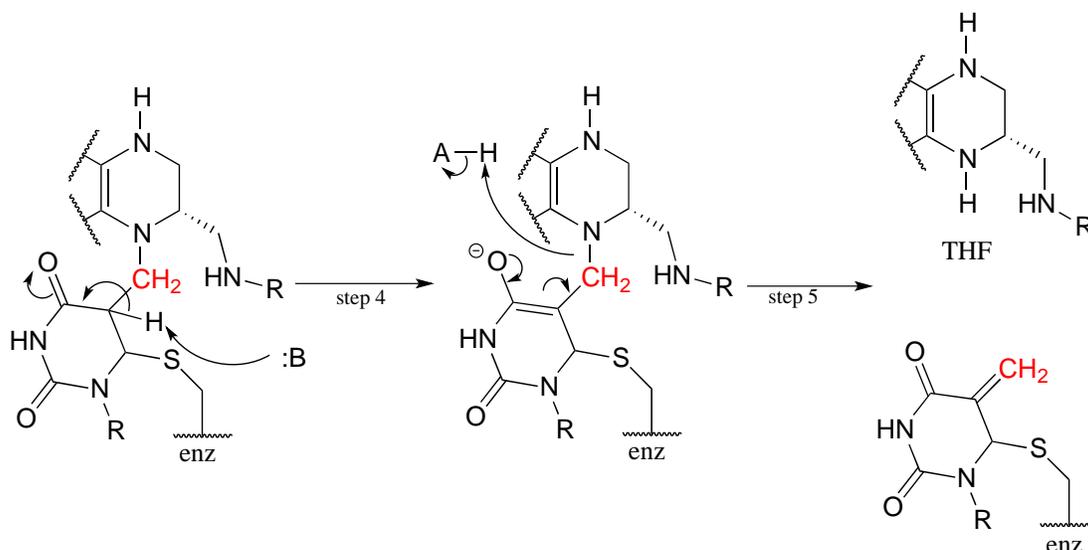
(*R* = ribose-5-phosphate)



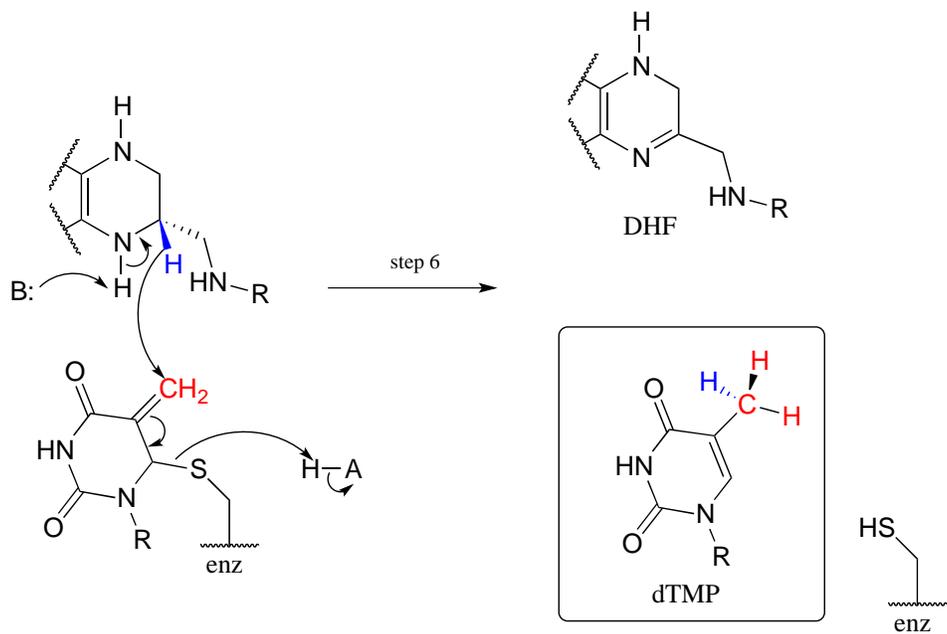
The reaction begins with the five-membered ring of CH₂-THF breaking apart to create an imine intermediate (step 1 below). The imine becomes an electrophile in a conjugate addition step (section 13.4, steps 2-3 below). Note that after step 1, the second ring in the pterin system of the coenzyme is abbreviated for clarity.



Next, tetrahydrofolate is eliminated in an E1cb elimination mechanism (steps 4 and 5). Notice that this is where the single carbon is transferred from methyl-THF to dUMP.



The final step in the mechanism is where it gets *really* interesting: a hydride ion is transferred from the tetrahydrofolate coenzyme to the methylene (CH₂) group on the deoxynucleotide substrate. Essentially, this is a *conjugated* S_N2 step with hydride as the nucleophile and the active site cysteine as the leaving group.



Key learning objectives for this chapter

After completing this chapter, you should be able to:

Understand how pyridoxal phosphate (PLP) acts as an 'electron sink' in a variety of reactions in amino acid metabolism.

Recognize and draw mechanisms for PLP-dependent transformations of the following types:

- racemization
- decarboxylation
- transamination
- retroaldol cleavage
- retro-Claisen cleavage
- β -elimination
- β -substitution
- γ -elimination
- γ -substitution

Recognize transformations - amino acid decarboxylation and transamination, for example - in which chemical steps occur that simply don't 'make sense' unless the electron sink role of PLP is taken into account.

Understand how the orientation of the substrate in relation to the plane formed by the conjugated π system of PLP is a major factor in catalysis of PLP-dependent reactions.

Understand how thiamine diphosphate (ThDP) acts as an 'electron sink' in a variety of reactions in which a bond to a carbonyl carbon is broken, and how these steps do not 'make sense' unless the electron sink role of ThDP is taken into account.

Recognize transformations for which ThDP is likely required, and be able to draw reasonable mechanisms for them.

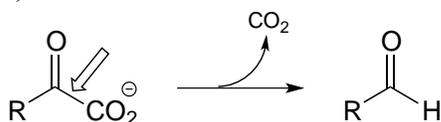
Understand how ThDP acts in tandem with lipoamide, flavin, and nicotinamide in the reaction catalyzed by pyruvate dehydrogenase.

Recognize folate in its various forms - DHF, THF, f-THF, CH₂-THF, and CH₃-THF - functions in a variety of one-carbon transfer steps. Be able to recognize the oxidation state of the carbon being transferred in a folate-dependent step.

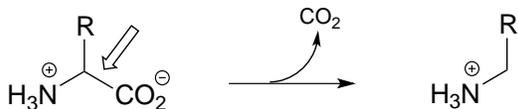
Problems

P17.1: Here is a chance to test your ability to recognize reactions catalyzed by enzymes using three coenzymes - thiamine diphosphate, pyridoxal phosphate, and folate - that we have studied in this chapter. For each generalized reaction, look carefully at the transformation that is taking place, and decide which of the three coenzymes is likely to be required. Then, draw the single mechanistic step by which the bond identified by an arrow is broken or formed. In the cases where a double bond is indicated, show the mechanistic step in which the σ bond is formed. In each case, your drawing should include the structure of the reactive part of the coenzyme, and should clearly show the role it plays in catalyzing the mechanistic step you are drawing.

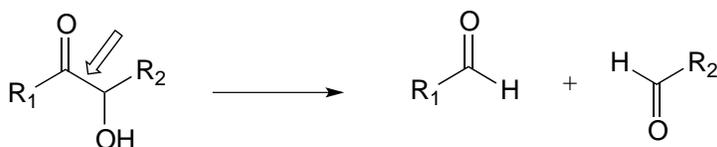
a)



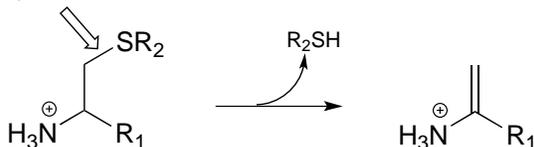
b)



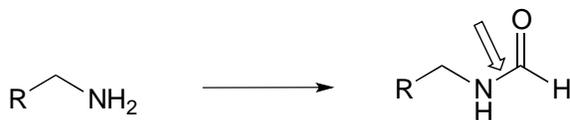
c)



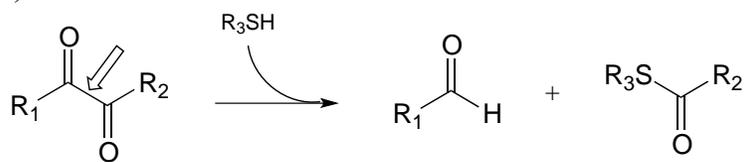
d)



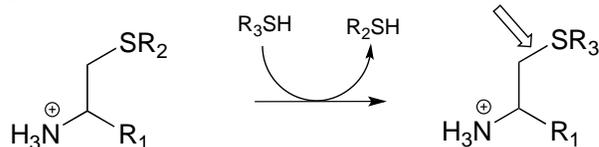
e)



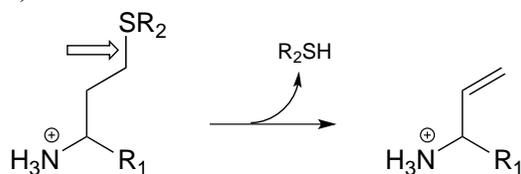
f)



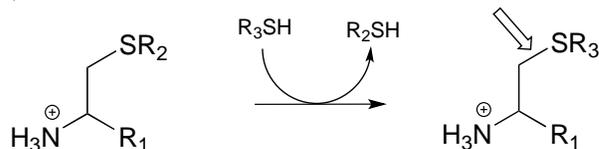
g)



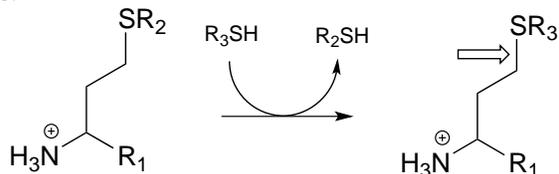
h)



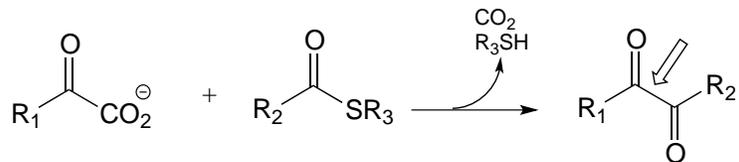
i)



j)



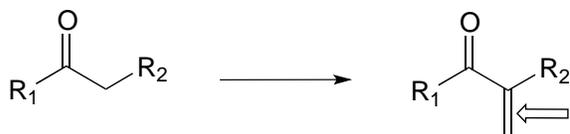
k)



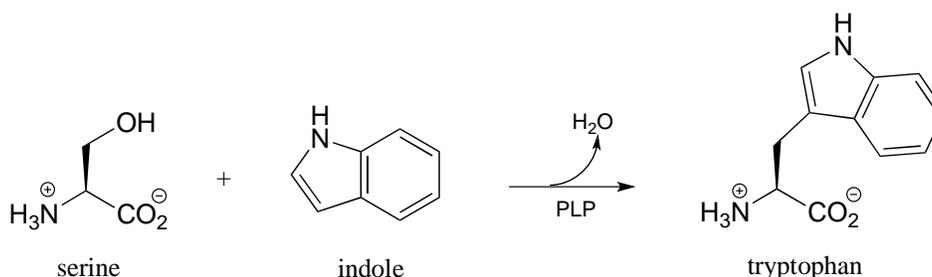
l)



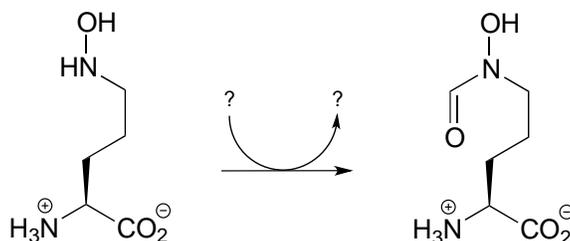
m)



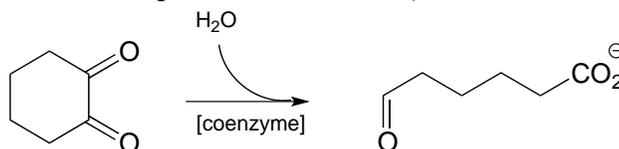
P17.2: The final step in the biosynthesis of the amino acid tryptophan is a PLP-dependent condensation between serine and indole, shown below (EC 4.2.1.20). The reaction mechanism involves steps that are familiar from this chapter, but also incorporates a reaction type we studied in chapter 14. Propose a mechanism.



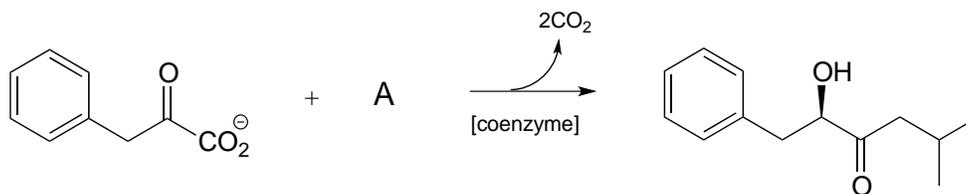
P17.3: Draw a reasonable mechanism for the following reaction, identifying the species denoted by question marks. (*Biochemistry* 2012, 51, 3059)



P17.4: Propose a mechanism for the reaction below, which is part of the anaerobic catabolism of alcohols in some species of bacteria. (*ChemBioChem* 2014, 15, 389)

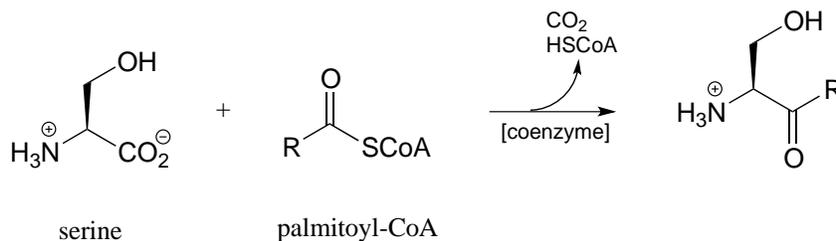


P17.5: Identify cosubstrate A and propose a mechanism for the reaction shown below, which was reported to occur in the thermophilic bacterium *Thermosporothrix hazakensis*. (*ChemBioChem* 2014 15, 527).

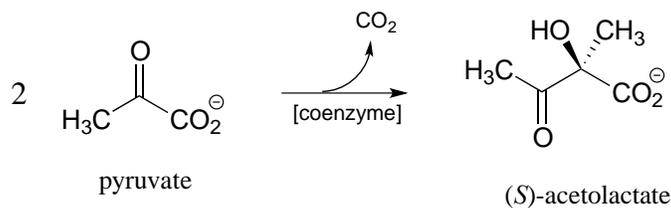


P17.6: Propose a mechanism for each of the reactions below, being sure to show the role played by the coenzyme (you need to determine which coenzyme is needed in each case).

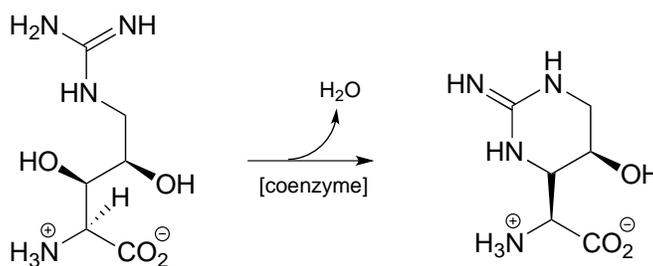
a)



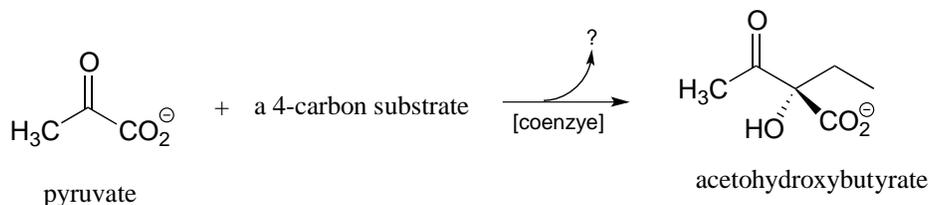
b) (E.C. 2.2.1.6)



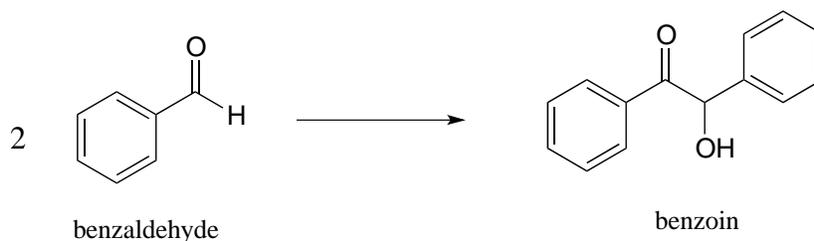
c) [Angew. Chem. Int. Ed. Engl. 2014, 53, 1943.](#)



P17.7: Acetoacetyl-CoA is formed in a coenzyme-dependent reaction between pyruvate and a 4-carbon compound. What is a likely second substrate, coenzyme, and by-product (indicated below with a question mark)?

**P17.8:**

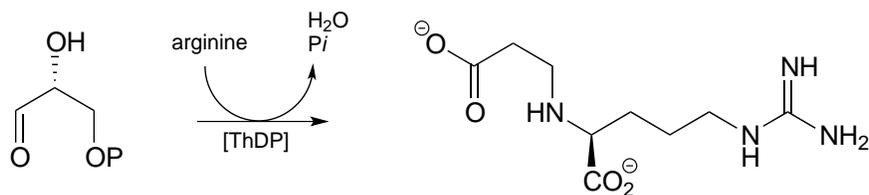
a) The 'benzoin condensation' reaction was discovered in the 19th century, and led eventually to a better understanding of ThDP-dependent reactions in the cell. In a traditional benzoin condensation reaction, cyanide ion (instead of ThDP) plays the role of electron acceptor. Enzyme-catalyzed benzoin condensation reactions are also known to occur in some bacteria: *Pseudomonas fluorescens*, for example, contains an enzyme that catalyzes the synthesis of (R)-benzoin.



a) Draw a mechanism for the enzyme-catalyzed (ThDP-dependent) benzoin condensation reaction.

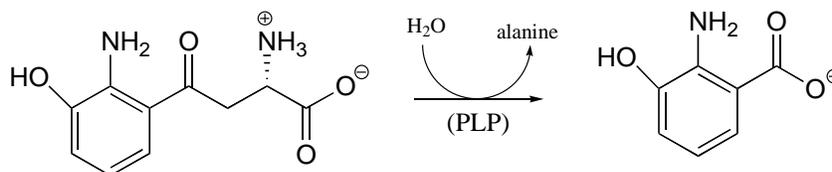
b) Draw a mechanism for the cyanide-catalyzed benzoin condensation reaction (non-enzymatic, basic conditions).

c) The following ThDP-reaction was recently reported to be part of the biosynthetic pathway for clavulanic acid, a compound that inhibits the action of β -lactamases (β -lactamases are bacterial enzymes that hydrolyze penicillin-based antibiotic drugs, rendering them ineffective). As is typical for ThDP-dependent reactions, the first step is addition of the ylide form of the coenzyme to the substrate carbonyl. The next steps are (in order): dehydration, tautomerization, elimination of phosphate, conjugate addition of arginine, and finally hydrolytic cleavage of the coenzyme-product bond. Draw out a complete mechanism that corresponds to this description.

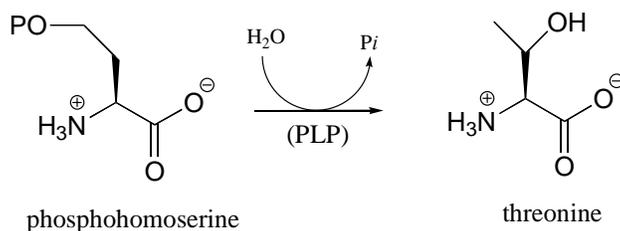


P17.9: Practice with PLP-dependent reactions:

a) Propose a mechanism for this reaction, which is part of the tryptophan degradation pathway (EC 3.7.1.3).

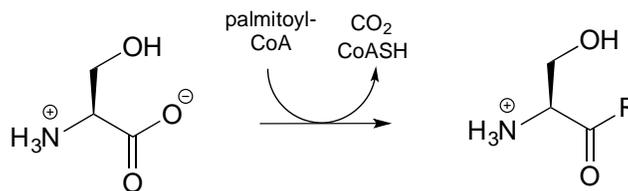


b) Propose a mechanism for the final step of the threonine biosynthesis pathway (EC 4.2.3.1)

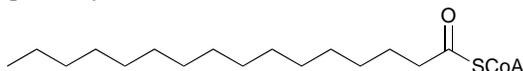


c) Propose a mechanism for the reaction catalyzed by aspartate β -decarboxylase (EC 4.1.1.12), which converts aspartate to alanine in a PLP-dependent reaction.

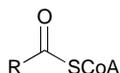
d) Sphingolipids are a type of membrane lipid found in the membranes of all eukaryotic cells, and are most abundant in the cells of central the central nervous system. Synthesis of sphingolipids involves the PLP-dependent reaction below, catalyzed by serine palmitoyl transferase (EC 2.3.1.50). Propose a mechanism.



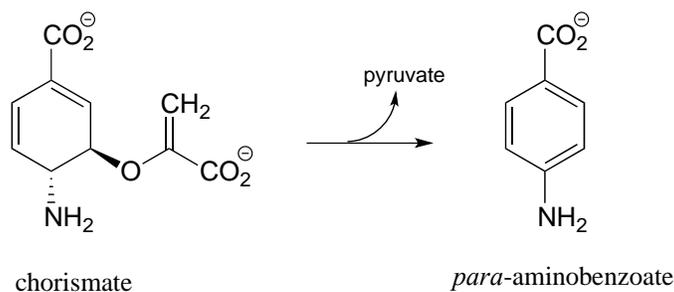
palmitoyl-CoA:



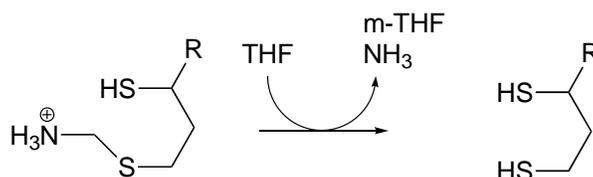
abbreviated:



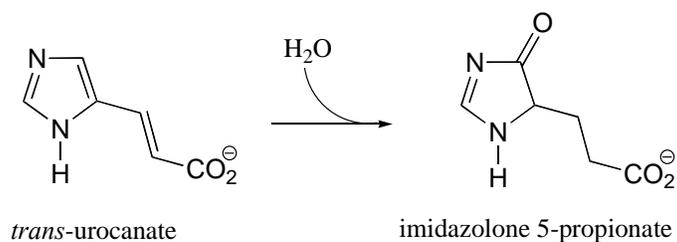
P17.10: As we saw in this chapter, PLP-dependent enzymes usually catalyze reactions involving amino acid substrates. Here is an exception, a PLP-dependent β -elimination reaction in the folate biosynthetic pathway (EC 4.1.3.38). Propose a mechanism for this reaction.



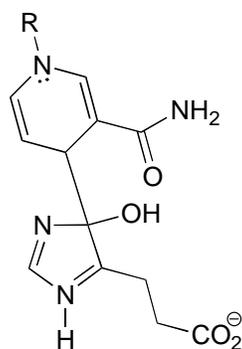
P17.11: The final step in the degradation pathway for the amino acid glycine (also known as the 'glycine cleavage system') is shown below. Propose a likely mechanism, given that evidence suggests that CH_2NH_2^+ is an intermediate.



P17.12: As we saw in chapter 15, the usual biochemical role of NAD^+ is to act as a hydride acceptor in dehydrogenation reactions. An exception is the reaction catalyzed by the histidine degradation pathway enzyme urocanase (EC 4.2.1.49).



In this reaction, NAD^+ acts as a catalytic, electron-sink coenzyme - it temporarily accepts electrons from a pi bond in the substrate, resulting in a covalent substrate-NAD adduct. This allows a key isomerization step to occur on the substrate through a protonation-deprotonation mechanism, followed by addition of water, cleavage of the substrate-NAD adduct to regenerate NAD^+ , and finally tautomerization to the product. Propose a mechanism that fits this description, and involves the intermediate below.

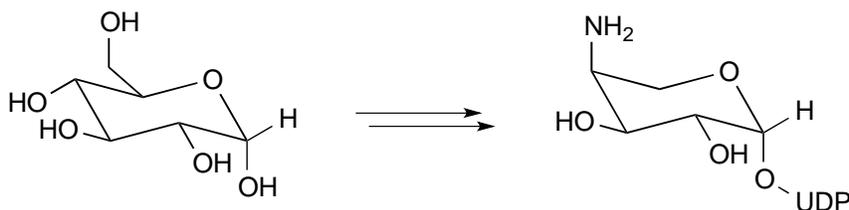


reaction intermediate

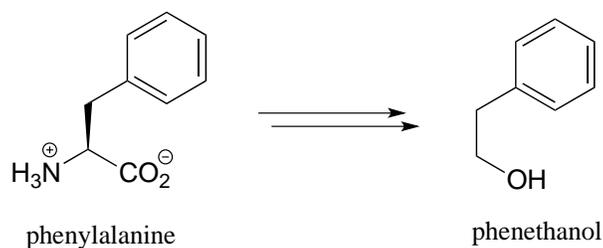
Pathway prediction problems

P17.13: Propose a multistep pathway for each of the following transformations. All involve at least one step requiring PLP, ThDP, or folate.

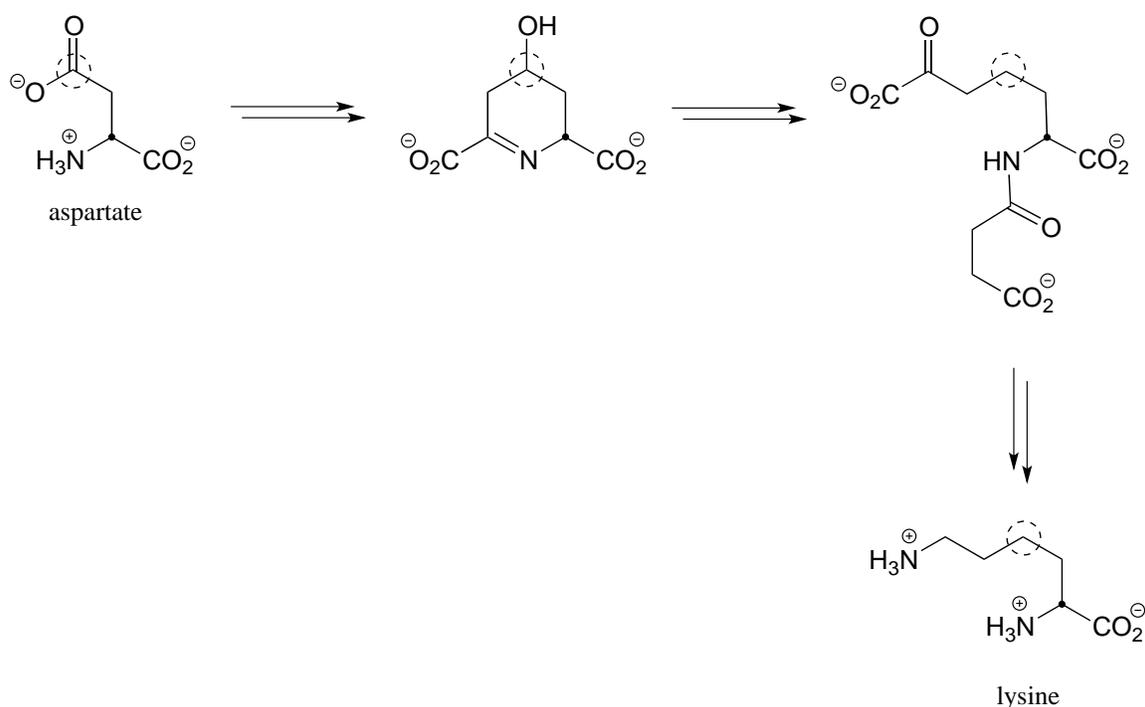
a) Below is portion of the biosynthesis of a modified membrane lipid in *Salmonella* and other pathogenic bacteria. The modified membrane confers antibiotic resistance to the bacterium.



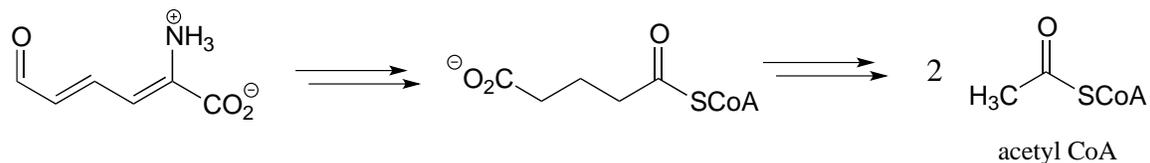
b) Below is the biosynthetic pathway for phenethanol in yeast. Phenethanol, which has a rose scent, is commonly used as a fragrance - this pathway has been proposed as a potential 'green' enzymatic synthesis to replace the traditional industrial synthesis, which uses toxic reagents.



c) Below is an incomplete pathway diagram for the biosynthesis of the amino acid lysine, starting from aspartate. Fill in the missing steps and reactants/coenzymes to complete the diagram. The solid dot and dashed circle are provided to help you to trace two of the carbons from substrate to product.

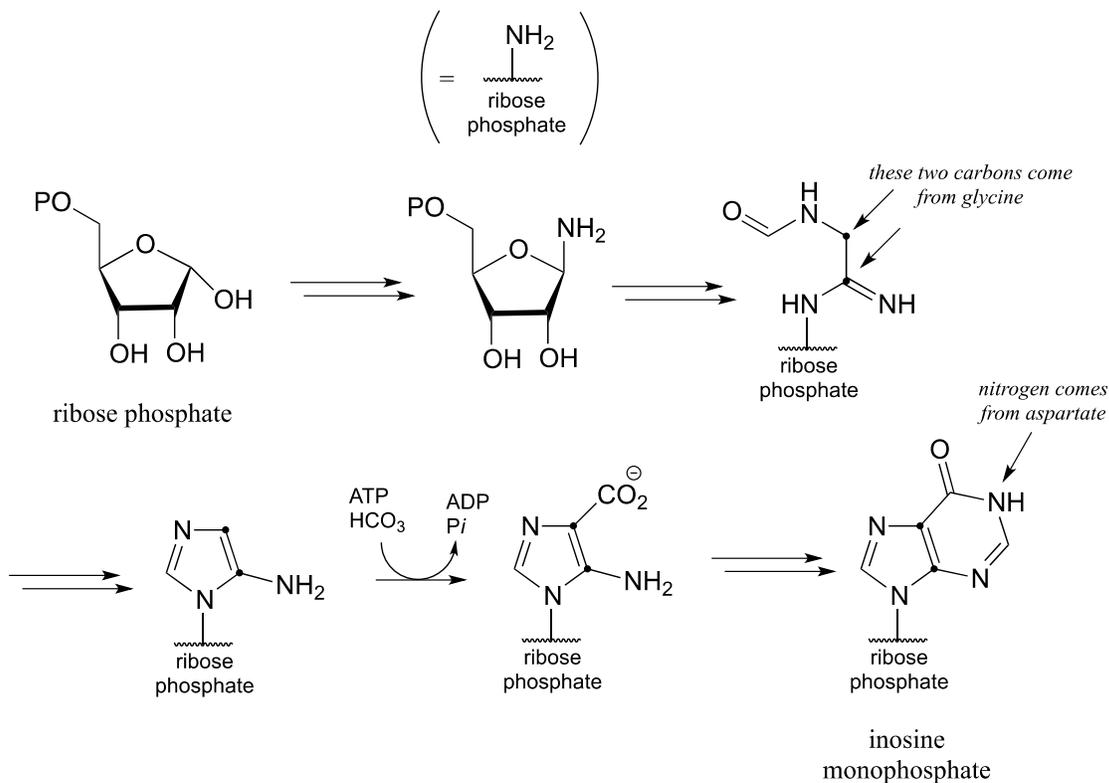


d) Below is the second half of the tryptophan degradation pathway. Fill in the missing steps and reactants/coenzymes to complete the diagram.



d) Below is an incomplete pathway diagram for the biosynthesis of inosine monophosphate, a precursor to the nucleotides adenosine monophosphate (AMP) and guanosine monophosphate (GMP). Fill in missing steps and reactants/coenzymes to

complete the diagram. Note that one enzymatic step is provided (this is a carboxylation reaction of a type that we have not studied).

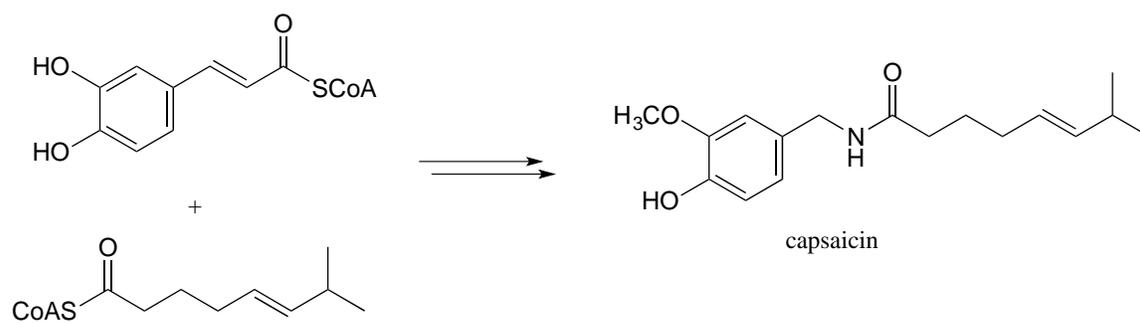


e) We begin our study of organic chemistry with a story about a hot pepper eating contest in Wisconsin (see the introduction the Chapter 1), and a compound called capsaicin which causes the 'hot' in hot peppers. As our last problem, let's try to predict some of the key steps in the biosynthesis of capsaicin.

Phase 1:



Phase 2:



Tables

List of tables:

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Table 2: Typical values for ^1H -NMR chemical shifts

Table 3: Typical values for ^{13}C -NMR chemical shifts

Table 4: Typical coupling constants in NMR

Table 5: The 20 common amino acids

Table 6: Structures of common coenzymes

Table 7: Representative acid constants

Table 8: Some common laboratory solvents, acids, and bases

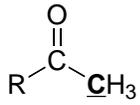
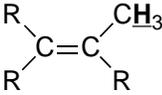
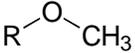
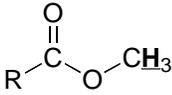
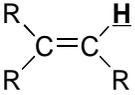
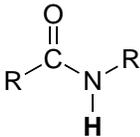
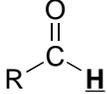
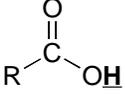
Table 9: Examples of common functional groups in organic chemistry

Table 1: Some characteristic absorption frequencies in IR spectroscopy

<u>Bond type</u>	<u>frequency (cm⁻¹)</u>
C-H alkanes	2950 – 2850
C-H alkenes	3080 – 3020
C-H aldehyde	~2900
C-H alkyne	~3300
alkyne triple bond	2250 – 2100 (s)
alkene double bond	1680 - 1620(s)
carbonyl, ketone	1725 – 1700 (s)
carbonyl, aldehyde	1740 – 1720 (s)
carbonyl, ester	1750 – 1730 (s)
carbonyl, acid	1725 – 1700 (s)
carbonyl, amide	1690 – 1650 (s)
O-H, alcohols	3600 – 3200 (s, broad)
O-H, acids	3000 – 2500 (broad)
C-O, alcohols, esters, ethers	1300 - 1000

s = strong absorbance

Table 2: Typical values for ^1H -NMR chemical shifts

<u>Hydrogen type</u>	<u>Chemical shift (ppm)</u>
RCH_3	0.9 - 1.0
RCH_2R	1.2 - 1.7
R_3CH	1.5 - 2.0
	2.0 - 2.3
	1.5 - 1.8
RNH_2	1 - 3
ArCH_3	2.2 - 2.4
$\text{R}-\text{C}\equiv\text{C}-\text{H}$	2.3 - 3.0
	3.7 - 3.9
	3.7 - 3.9
ROH	1 - 5
	3.7 - 6.5
	5 - 9
ArH	6.0 - 8.7
	9.5 - 10.0
	10 - 13

Chemical shift values are in parts per million (ppm) relative to tetramethylsilane.

Table 3: Typical values for ^{13}C -NMR chemical shifts

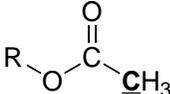
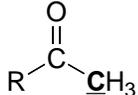
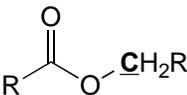
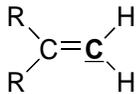
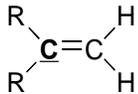
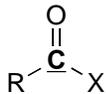
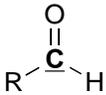
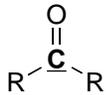
<u>Carbon type</u>	<u>Chemical shift (ppm)</u>
$\text{R}\underline{\text{C}}\text{H}_3$	13 - 16
$\text{R}\underline{\text{C}}\text{H}_2\text{R}$	16 - 25
$\text{R}_3\underline{\text{C}}\text{H}$	25 - 35
	18 - 22
	28 - 32
$\text{R}\underline{\text{C}}\text{H}_2\text{NHR}$	35 - 45
$\text{R}\underline{\text{C}}\text{H}_2\text{OH}$	50 - 65
$\text{R}-\text{C}\equiv\underline{\text{C}}-\text{R}$	65 - 70
$\text{RO}\underline{\text{C}}\text{H}_2\text{R}$	50 - 75
	50 - 75
	115 - 120
	125 - 140
aromatic carbon	125 - 150
 (carboxylic acid derivatives)	165 - 185
	190 - 200
	200 - 220

Table 4: Typical coupling constants in NMR

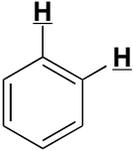
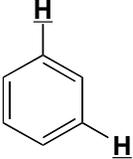
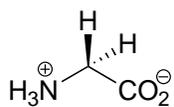
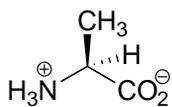
<u>H-H coupling</u>	<u>J (Hz)</u>	<u>C-H coupling</u>	<u>J (Hz)</u>
$\begin{array}{c} \text{R} \quad \text{R} \\ \quad \\ \text{R}-\text{C}-\text{C}-\text{R} \\ \quad \\ \text{H} \quad \text{H} \end{array}$	6 - 8	$\begin{array}{c} \text{R} \\ \\ \text{R}-\text{C}-\text{H} \\ \\ \text{R} \end{array}$	125 - 130
$\begin{array}{c} \text{R} \quad \text{O} \\ \quad \\ \text{R}-\text{C}-\text{C}-\text{H} \\ \\ \text{H} \end{array}$	2 - 3	$\begin{array}{c} \text{R} \quad \text{H} \\ \diagdown \quad / \\ \text{C}=\text{C} \\ / \quad \diagdown \\ \text{R} \quad \text{R} \end{array}$	150 - 170
$\begin{array}{c} \text{H} \quad \text{R} \\ \diagdown \quad / \\ \text{C}=\text{C} \\ / \quad \diagdown \\ \text{R} \quad \text{H} \end{array}$	12 - 18		
$\begin{array}{c} \text{R} \quad \text{R} \\ \diagdown \quad / \\ \text{C}=\text{C} \\ / \quad \diagdown \\ \text{H} \quad \text{H} \end{array}$	6 - 12		
$\begin{array}{c} \text{R} \quad \text{H} \\ \diagdown \quad / \\ \text{C}=\text{C} \\ / \quad \diagdown \\ \text{R} \quad \text{H} \end{array}$	0 - 2		
	6 - 10		
	1 - 3		

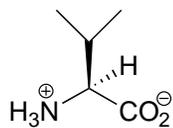
Table 5: The 20 common amino acids



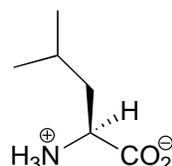
Glycine
(Gly, G)



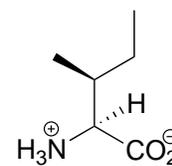
Alanine
(Ala, A)



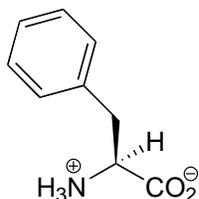
Valine
(Val, V)



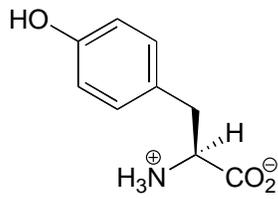
Leucine
(Leu, L)



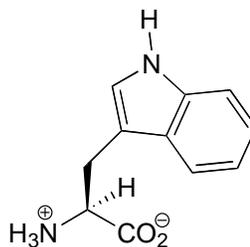
Isoleucine
(Ile, I)



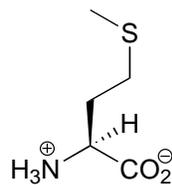
Phenylalanine
(Phe, F)



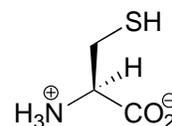
Tyrosine
(Tyr, Y)



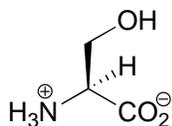
Tryptophan
(Trp, W)



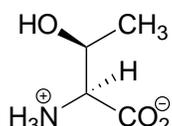
Methionine
(Met, M)



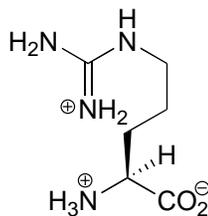
Cysteine
(Cys, C)



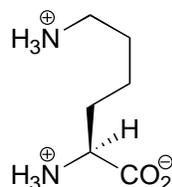
Serine
(Ser, S)



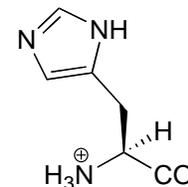
Threonine
(Thr, T)



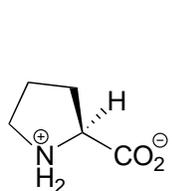
Arginine
(Arg, R)



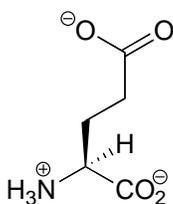
Lysine
(Lys, K)



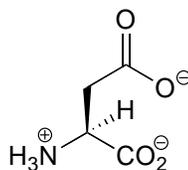
Histidine
(His, H)



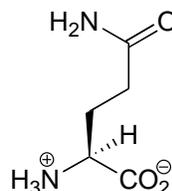
Proline
(Pro, P)



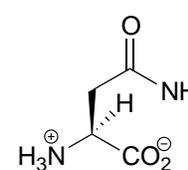
Glutamate
(Glu, E)



Aspartate
(Asp, D)

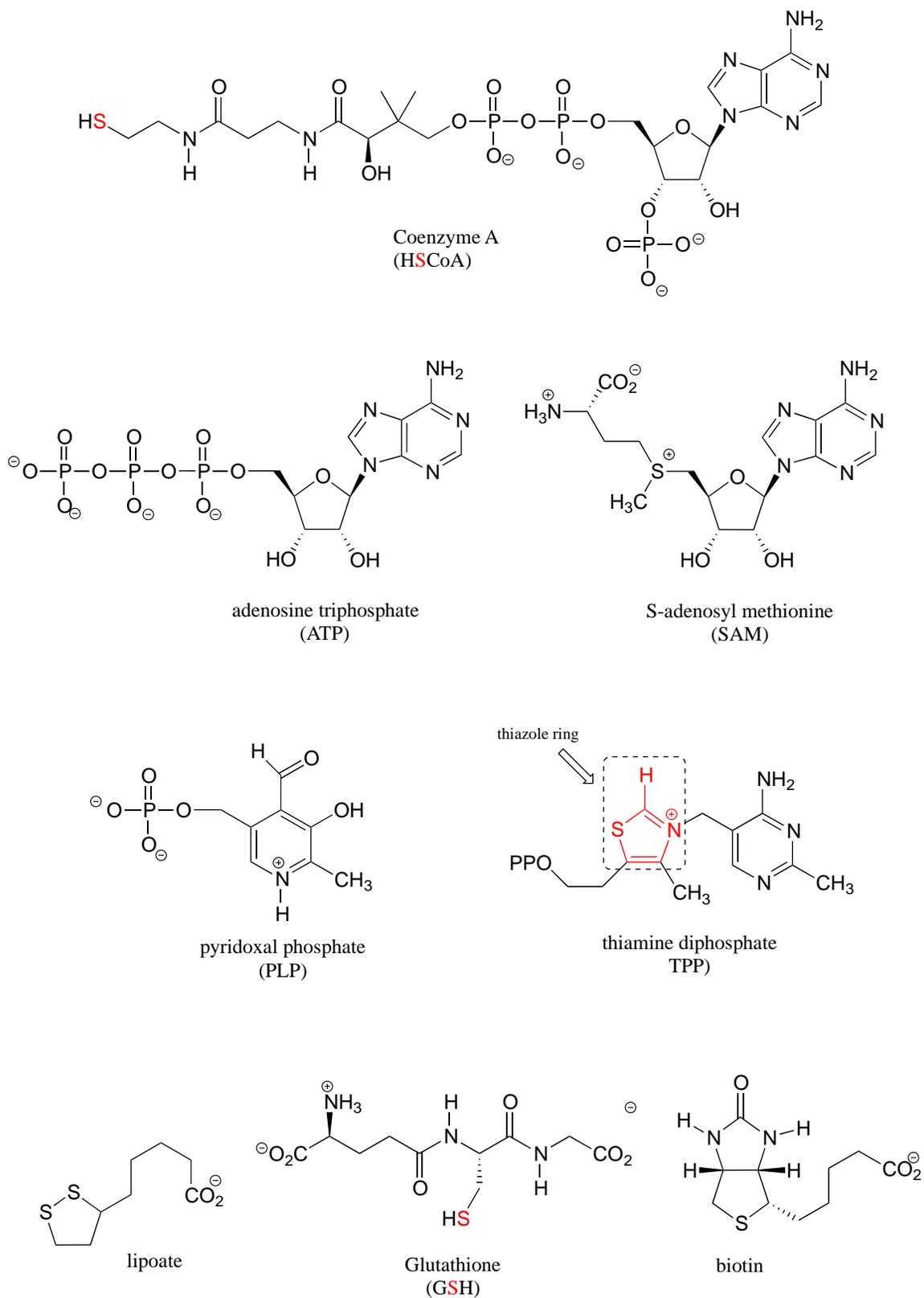


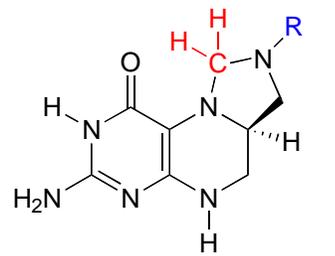
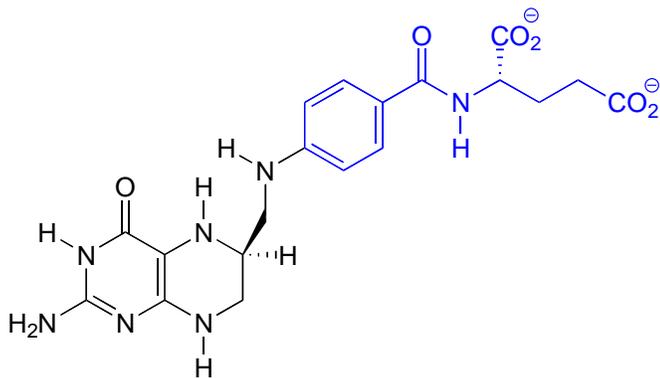
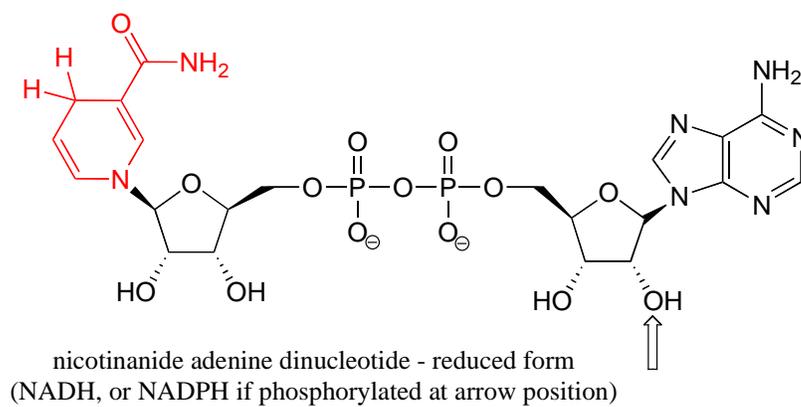
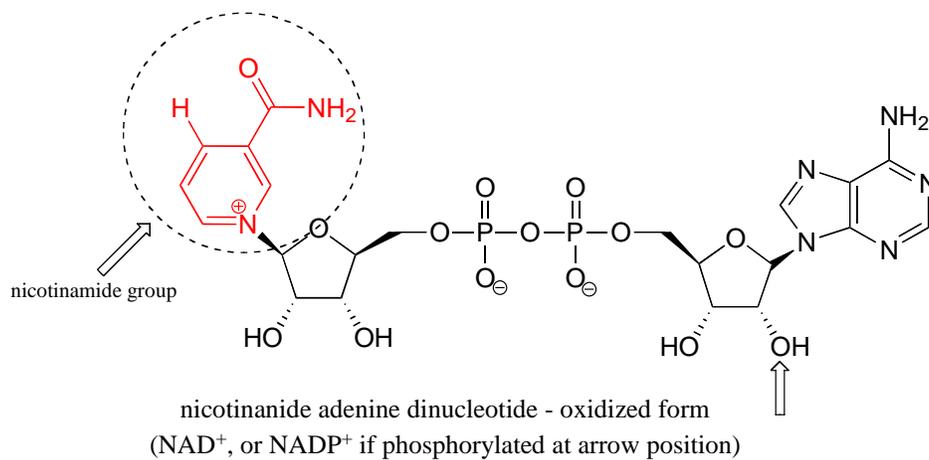
Glutamine
(Gln, Q)

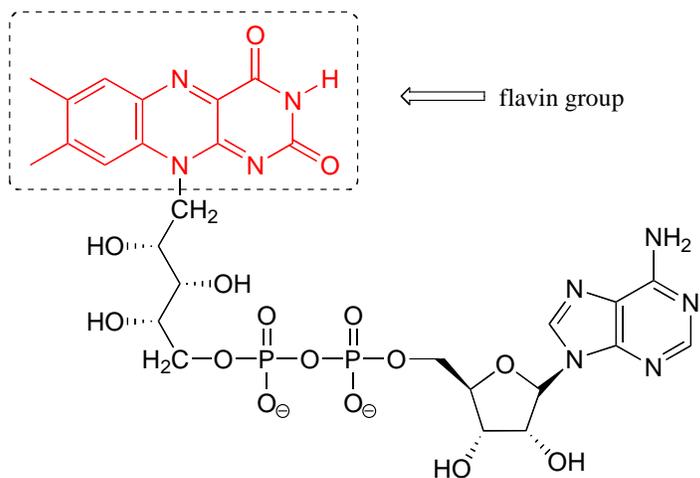


Asparagine
(Asn, N)

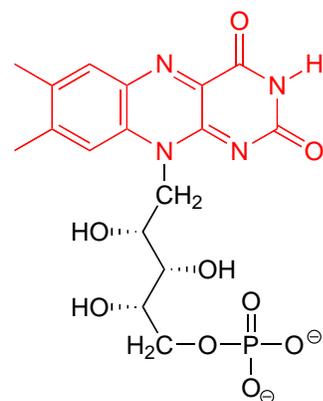
Table 6: Structures of common coenzymes



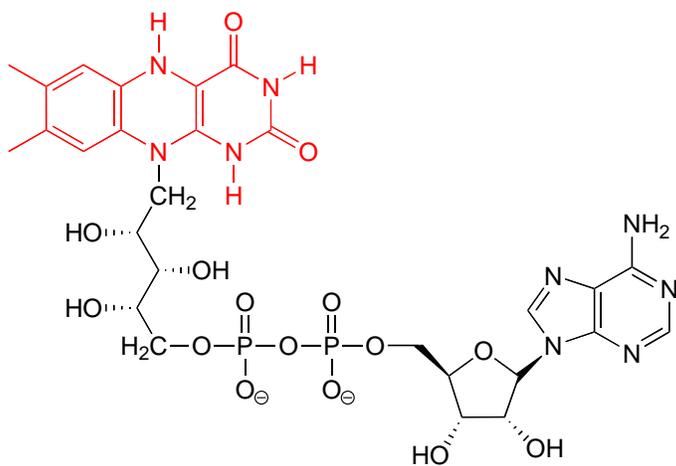




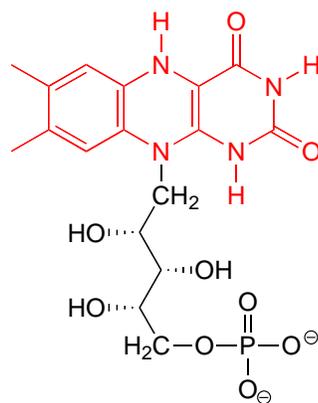
flavin adenine dinucleotide, oxidized form
(FAD)



flavin mononucleotide, oxidized form
(FMN)

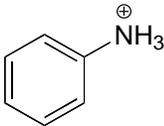
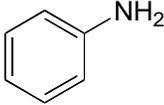
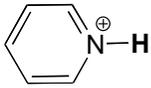


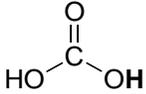
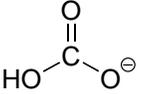
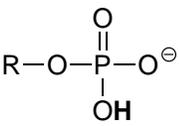
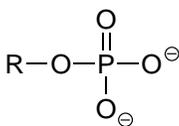
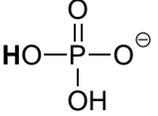
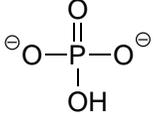
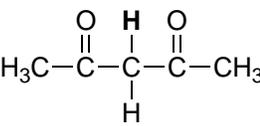
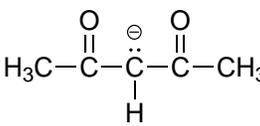
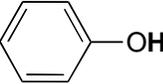
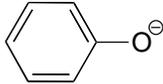
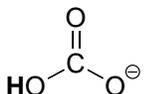
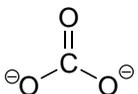
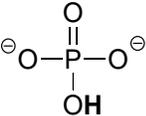
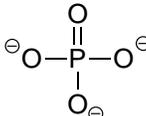
flavin adenine dinucleotide, reduced form
(FADH₂)



flavin mononucleotide, reduced form
(FMNH₂)

Table 7: Representative acid constants.

<u>acid</u>	<u>pK_a</u>	<u>conjugate base</u>
$\begin{array}{c} \text{O} \\ \parallel \\ \text{HO}-\text{S}-\text{OH} \\ \parallel \\ \text{O} \end{array}$ <p>sulfuric acid</p>	-10	$\begin{array}{c} \text{O} \\ \parallel \\ \text{HO}-\text{S}-\text{O}^{\ominus} \\ \parallel \\ \text{O} \end{array}$
HCl	-7	Cl ⁻
H ₃ O ⁺	0 ⁽ⁱⁱⁱ⁾	H ₂ O
$\begin{array}{c} \text{O} \\ \parallel \\ \ominus\text{O}-\text{N}^{\oplus}-\text{OH} \end{array}$ <p>nitric acid</p>	-1.4	$\begin{array}{c} \text{O} \\ \parallel \\ \ominus\text{O}-\text{N}^{\oplus}-\text{O}^{\ominus} \end{array}$
$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{O}-\text{P}-\text{OH} \\ \\ \text{OH} \end{array}$	1.0 ⁽ⁱ⁾	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{O}-\text{P}-\text{O}^{\ominus} \\ \\ \text{OH} \end{array}$
$\begin{array}{c} \text{O} \\ \parallel \\ \text{HO}-\text{P}-\text{OH} \\ \\ \text{OH} \end{array}$ <p>phosphoric acid</p>	2.2 ⁽ⁱⁱ⁾	$\begin{array}{c} \text{O} \\ \parallel \\ \text{HO}-\text{P}-\text{O}^{\ominus} \\ \\ \text{OH} \end{array}$
HF	3.2	F ⁻
	4.6	
$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{OH} \end{array}$	4-5	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{O}^{\ominus} \end{array}$
 <p>pyridinium</p>	5.3 ⁽ⁱⁱ⁾	 <p>pyridine</p>

<u>acid</u>	<u>pK_a</u>	<u>conjugate base</u>
 carbonic acid	6.4	 bicarbonate
 R-O-P(=O)(OH)-O [⊖]	6.5(i)	 R-O-P(=O)(O [⊖])-O [⊖]
 HO-P(=O)(OH)-O [⊖]	7.2(ii)	 [⊖] O-P(=O)(OH)-O [⊖]
 H ₃ C-C(=O)-C(H)-C(=O)-CH ₃	9.0	 H ₃ C-C(=O)-C(H) [⊖] -C(=O)-CH ₃
HCN	9.2	CN ⁻
 ammonium	9.2	NH ₃ ammonia
 phenol	9.9(ii)	 phenolate
 bicarbonate	10.3(ii)	 carbonate
RSH	10-11	RS ⁻
RNH ₃ ⁺	10 -11	RNH ₂
 [⊖] O-P(=O)(OH)-O [⊖]	12.3(i)	 [⊖] O-P(=O)(O [⊖])-O [⊖]
H ₂ O	14.0(iii)	OH ⁻

<u>acid</u>	<u>pK_a</u>	<u>conjugate base</u>
$\begin{array}{c} \text{O} \\ \\ \text{R}-\text{C}-\text{NH}_2 \end{array}$	17	$\begin{array}{c} \text{O} \\ \\ \text{R}-\text{C}-\text{NH}^- \end{array}$
RCH ₂ OH	16	RCH ₂ O ⁻
$\begin{array}{c} \text{O} \\ \\ \text{R}-\text{C}-\text{C}-\text{R} \\ \quad \\ \text{H} \quad \text{H} \end{array}$	19-20	$\begin{array}{c} \text{O} \\ \\ \text{R}-\text{C}-\text{C}^- -\text{R} \\ \\ \text{H} \end{array}$
RCCH terminal alkyne	25	RCC ⁻
H ₂	35	H ⁻
NH ₃ ammonia	38	NH ₂ ⁻

All pK_a values, unless otherwise noted, are taken from March, Jerry, Advanced Organic Chemistry, Fourth Edition, Wiley, New York, 1992.

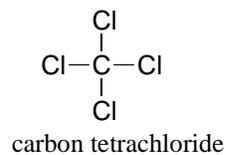
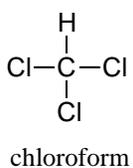
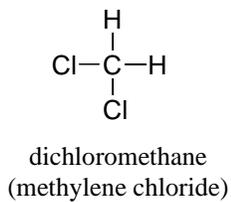
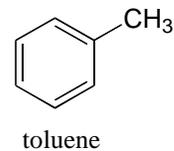
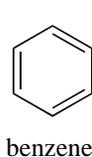
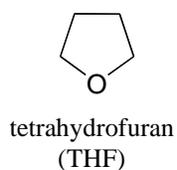
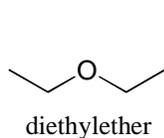
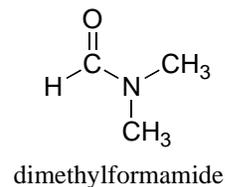
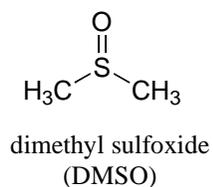
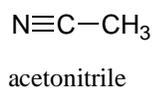
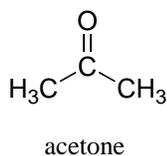
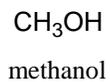
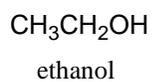
(i) Silva, J.J.R. Fraústo da, The Biological Chemistry of the Elements: the Inorganic Chemistry of Life, 2nd Edition, Oxford, New York, 2001.

(ii) Lide, David R. (ed.) The CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1995.

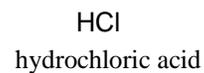
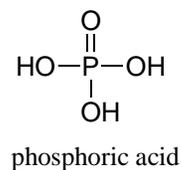
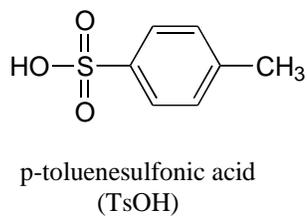
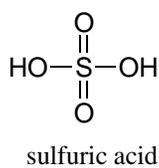
(iii) Although the accepted pK_a of water is 14, it is sometimes incorrectly listed as 15.7 in Organic Chemistry textbooks. Likewise, the accepted pK_a of hydronium ion is 0, although it is sometimes incorrectly listed as -1.7. See [J. Chem Ed. 2017, 94, 690](#) for more discussion of this discrepancy.

Table 8: Some common laboratory solvents, acids, and bases

Solvents

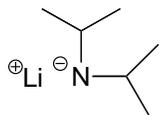


Acids

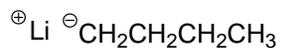


Bases

very strong bases:



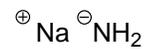
lithium diisopropylamide
(LDA)



N-butyllithium

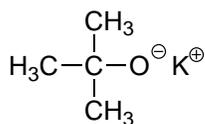


sodium hydride

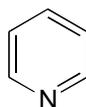


sodium amide

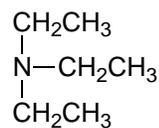
weaker bases:



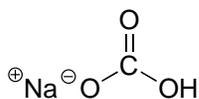
potassium *tert*-butoxide



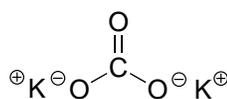
pyridine



triethylamine



sodium bicarbonate
 NaHCO_3



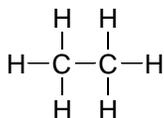
potassium carbonate
 K_2CO_3



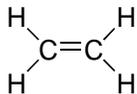
sodium hydroxide

Table 9: Examples of common functional groups in organic chemistry

alkane



alkene



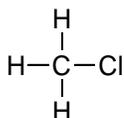
alkyne



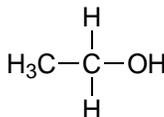
aromatic hydrocarbon



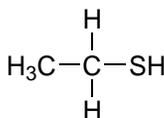
alkyl halide



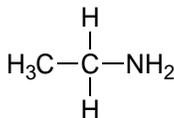
alcohol



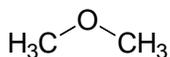
thiol



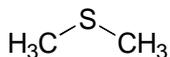
amine



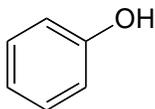
ether



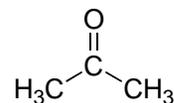
sulfide



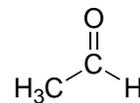
phenol



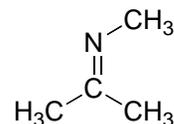
ketone



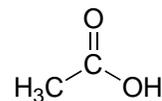
aldehyde



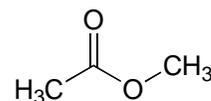
imine (Schiff base)



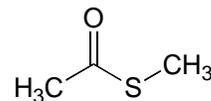
carboxylic acid



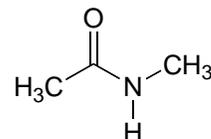
ester



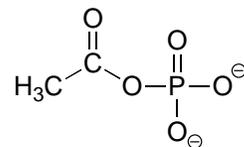
thioester



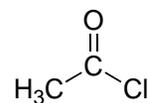
amide



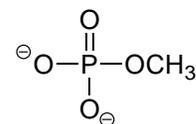
acyl phosphate



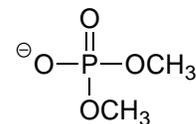
acid chloride



phosphate ester



phosphate diester



Appendix 1: Enzymatic reactions by metabolic pathway and EC number

The EC (European Commission) number is a classification system for enzymes, organized by the type of reaction catalyzed. You can use the list below to search this textbook for information about the reaction catalyzed by a given enzyme. You can also use the EC numbers to search for information in databases such as SwissProt Enzyme Nomenclature Database (<http://enzyme.expasy.org>) or the BRENDA Comprehensive Enzyme Information System (<http://www.brenda-enzymes.org>).

Glycolysis

Hexose kinase (EC 2.7.1.1)
Phosphoglucose isomerase (EC 5.3.1.9)
Phosphofructokinase (EC 2.7.1.56)
Fructose 1,6-bisphosphate aldolase (EC 4.1.2.13)
Triose phosphate isomerase (5.3.1.1)
Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12)
Phosphoglycerate kinase (EC 2.7.2.3)
Phosphoglycerate mutase (EC 5.4.2.1)
Enolase (EC 4.2.1.11)
Pyruvate kinase (EC 2.7.1.40)

2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.1.2.14)

Gluconeogenesis

Pyruvate carboxylase (EC 6.4.1.1)
Phosphoenolpyruvate carboxykinase (EC 4.1.1.32)
Phosphoglycerate kinase (EC 2.7.2.3)
Fructose 1,6-bisphosphatase (EC 3.1.3.11)
Glucose 6-phosphatase (EC 3.1.3.9)

Citric Acid (TCA) Cycle

Pyruvate dehydrogenase complex (EC 1.2.4.1)
Citrate synthase (EC 2.3.3.8)
Aconitase (EC 4.2.1.3)
Isocitrate dehydrogenase (EC 1.1.1.42)
 α -ketoglutarate dehydrogenase complex:
 Oxoglutarate dehydrogenase (EC 1.2.4.2)
 Dihydrolipoyl succinyltransferase (EC 2.3.1.61)
 Dihydrolipoyl dehydrogenase (EC 1.8.1.4)
Succinyl CoA synthetase (EC 6.2.1.4)
Succinate dehydrogenase (EC 1.3.5.1)
Fumarase (EC 4.2.1.2)
Malate dehydrogenase (EC 1.1.1.37)

Fermentation

Pyruvate decarboxylase (EC 4.1.1.1)
Alcohol dehydrogenase (EC 1.1.1.1)
Lactate dehydrogenase ((EC 1.1.1.27)

Pentose phosphate pathway/Calvin Cycle

Glucose 6-phosphate dehydrogenase (EC 1.1.1.49)
Gluconolactonase (EC 3.1.1.17)
6-Phosphogluconate dehydrogenase (EC 1.1.1.43)
Phosphopentose epimerase (EC 5.1.3.1)
Phosphopentose isomerase (EC 5.3.1.6)
Transketolase (EC 2.2.1.1)
Transaldolase (EC 2.2.1.2)
Rubisco (EC 4.1.1.39)
Aldolase (EC 4.1.2.13)

Fatty acid oxidation

Acyl CoA synthetase (EC 6.2.1.1)
Carnitine acyltransferase (EC 2.3.1.21)
Acyl CoA dehydrogenase (eg. EC 1.3.99.13)
Enoyl CoA hydratase (eg. 4.2.1.74)
3-hydroxyacyl CoA dehydrogenase (EC 1.1.1.35)
 β -Keto thiolase (EC 2.3.1.16)

Unsaturated fatty acids:*cis*-Enoyl-CoA isomerase (eg. EC 5.3.3.8)

2,4-Dienoyl CoA reductase (EC 1.3.1.34)

Glycerol phosphate dehydrogenase (EC 1.1.1.8)

Fatty acid biosynthesis

Acetyl CoA carboxylase (EC 6.4.1.2)

Acyl CoA synthetase (EC 6.2.1.1)

ACP transacylase (EC 2.3.1.38)

 β -ketoacyl-ACP synthase (EC 2.3.1.41) β -ketoacyl-ACP hydrogenase (EC 1.1.1.35)

3-hydroxyacyl dehydratase (EC 4.2.1.58)

Enoyl-ACP reductase (EC 1.3.1.10)

Acyl-CoA dehydrogenase (EC 1.3.99.3)

Monoacylglycerol acyltransferase (EC 2.3.1.22)

Isoprenoid biosynthesis***Mevalonate pathway (from acetyl CoA):***

Acetoacetyl CoA acetyltransferase (EC 2.3.1.9)

3-hydroxy-3-methylglutaryl-CoA synthase (EC 2.3.3.10)

HMG-CoA reductase (EC 1.1.1.34)

Mevalonate kinase (EC 2.7.1.36)

Phosphomevalonate kinase (EC 2.7.4.2)

Mevalonate diphosphate decarboxylase (EC 4.1.1.33)

Deoxyxylulose pathway (from pyruvate and glyceraldehyde phosphate):

Deoxyxylulose phosphate synthase (EC 2.2.1.7)

Deoxyxylulose phosphate reductoisomerase (EC 1.1.1.267)

MEP cytidylyltransferase (EC 2.7.7.60)

CDP-ME kinase (EC 2.7.1.148)

Methylethritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12)

other:

isopentenyl diphosphate isomerase (EC 5.3.3.2)
 geranyl diphosphate synthase (EC 2.5.1.1)
 farnesyl diphosphate synthase (EC 2.5.1.10)
 squalene synthase (EC 2.5.1.21)
 oxidosqualene cyclase (EC 5.4.99.7)

DMAPP-tryptophan synthase (EC 2.5.1.34)

Deoxyribonucleotide biosynthesis

PRPP synthetase (EC 2.7.6.1)

UMP (from ammonia, bicarbonate, and aspartate):

Carbamoyl phosphate synthase (EC 6.3.5.5)
 Aspartate carbamoyltransferase (EC 2.1.3.2)
 Dihydroorotase (EC 2.5.2.3) 4
 Dihydroorotate dehydrogenase (EC 1.3.3.1)
 Orotate phosphoribosyltransferase (EC 2.4.2.10)
 Orotidine monophosphate decarboxylase (EC 4.1.1.23)

TMP (from dUMP):

Thymidylate synthase (EC 2.1.1.45)

CTP: (from UTP):

CTP synthase (EC 6.3.4.2)

IMP (from PRPP):

Glutamine phosphoribosyl amidotransferase (EC 2.4.2.14)
 Glycinamide ribonucleotide synthetase (EC 6.3.4.13)
 GAR transformylase (EC 2.1.2.2)
 FGAM synthetase (EC 6.3.5.3)
 Aminoimidazole ribonucleotide synthetase (EC 6.3.3.1)
 Aminoimidazole ribonucleotide carboxylase (EC 4.2.1.1)
 SAICAR synthetase (EC 6.3.2.6)
 Adenylosuccinate lyase (EC 4.3.2.2)
 AICAR transformylase (EC 2.1.2.3)
 IMP cyclohydrolase (EC 3.5.4.10)

AMP (from IMP):

Adenylosuccinate synthetase (EC 6.3.4.4)
 Adenylosuccinate lyase (EC 4.3.2.2)

GMP (from IMP):

IMP dehydrogenase (EC 1.1.1.205)

GMP synthetase (EC 6.3.5.2)

Deoxyribonucleotides:

Ribonucleotide reductase (EC 1.17.4.1)

Nucleotide degradation

Cytidine (to uridine):

Cytidine deaminase (EC 3.5.4.5)

Uridine (to malonyl CoA):

Uridine phosphorylase (EC 2.4.2.3)

Dihydropyrimidine dehydrogenase (EC 1.3.1.2)

Dihydropyrimidase (EC 3.5.2.2)

β -ureidopropionase (EC 3.5.1.6)

Thymidine (to succinyl CoA):

Thymidine phosphorylase (EC 2.4.2.4)

Dihydropyrimidine dehydrogenase (EC 1.3.1.2)

Dihydropyrimidase (EC 3.5.2.2)

β -ureidopropionase (EC 3.5.1.6)

Adenosine (to uric acid):

Adenosine deaminase (EC 3.5.4.4)

Purine nucleoside phosphorylase (EC 2.4.2.1)

Xanthine oxidase (EC 1.17.1.4; EC 1.17.3.2)

Guanosine (to uric acid):

Purine nucleoside phosphorylase (EC 2.4.2.1)

Guanine deaminase (EC 3.5.4.3)

Xanthine oxidase (EC 1.17.1.4; EC 1.17.3.2)

Amino acid biosynthesis**Alanine** (*from pyruvate*):

Alanine transaminase (EC 2.6.1.2)

Alanine racemase (PLP-dependent) (EC 5.1.1.1)

Aspartate (*from oxaloacetate*):

Aspartate transaminase (EC 2.6.1.1)

Glutamate (*from α -ketoglutarate*):

Glutamate transaminase (EC 2.6.1.1)

Glutamine (*from glutamate*):

Glutamine synthase (EC 6.3.1.2)

Asparagine (*from aspartate*):

Asparagine synthase (EC 6.3.5.4)

Arginine (*from glutamate via ornithine*):

N-acetylglutamate synthase (EC 2.3.1.1)

Acetylglutamate kinase (EC 2.7.2.8)

N-acetyl- γ -glutamyl-phosphate reductase (1.2.1.38)

Acetylornithine transaminase (EC 2.6.1.11)

Acetylornithine deacetylase (EC 3.5.1.16)

*then ornithine to arginine via urea cycle***Proline** (*from glutamate*):

Glutamate-5-kinase (EC 2.7.2.11)

Glutamate-5-semialdehyde dehydrogenase (EC 1.2.1.41)

Pyrroline-5-carboxylate reductase (EC 1.5.1.2)

Serine (*from 3-phosphoglycerate*):

Phosphoglycerate dehydrogenase (EC 1.1.1.95)

Phosphoserine transaminase (EC 2.6.1.52)

Phosphoserine phosphatase (EC 3.1.3.3)

Cysteine (*from serine*):Cystathionine β -synthase (EC 4.2.1.22)Cystathionine γ -lyase (EC 4.4.1.1)... *or (in bacteria)* :

O-acetylserine sulfhydrylase (EC 2.5.1.47)

Glycine (*from serine*):

Serine hydroxymethyltransferase (EC 2.1.2.1)

Lysine (*from aspartate*):

Aspartate kinase (EC 2.7.2.4) P16.3

Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)

Dihydrodipicolinate synthase (EC 4.2.1.52)

Dihydrodipicolinate reductase (EC 1.3.1.26)

Tetrahydropyridine-2-carboxylate *N*-succinyltransferase (EC 2.3.1.117)

Succinyl-diaminopimelate transaminase (EC 2.6.1.17)

Succinyl-diaminopimelate desuccinylase (EC 3.5.1.18)

Diaminopimelate decarboxylase (EC 4.1.1.20)

Methionine (*from aspartate via homoserine*):

Aspartate kinase (EC 2.7.2.4)

Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)

Homoserine dehydrogenase (EC 1.1.1.3)

Homoserine transsuccinylase (EC 2.3.1.46)

Cystathionine α -synthase (EC 4.2.1.22)Cystathionine β -lyase (EC 4.4.1.8)

Methionine synthase (EC 2.1.1.13)

Threonine (*from homoserine*):

Homoserine kinase (EC 2.7.1.39)

Threonine synthase (EC 4.2.3.1)

Isoleucine, leucine, valine (*from pyruvate*):

Acetolactate synthase (EC 2.2.1.6)

Ketol acid reductoisomerase (EC 1.1.1.86)

Dihydroxyacid dehydratase (EC 4.2.1.9)

Isoleucine:

Branched-chain-amino-acid transaminase (EC 2.6.1.42)

Valine:

Valine—pyruvate transaminase (EC 2.6.1.66)

Leucine:

2-isopropylmalate synthase (EC 2.3.3.13)

Isopropylmalate isomerase (EC 4.2.1.33)

3-isopropylmalate dehydrogenase (EC 1.1.1.85)

Leucine transaminase (EC 2.6.1.6)

Aromatic amino acids***Erythrose-4P to chorismate:***

DAHP synthase (EC 2.5.1.54)
 Dehydroquinate synthase (EC 4.2.3.4)
 Dehydroquinate dehydratase (EC 4.2.1.10)
 Shikimate dehydrogenase (EC 1.1.1.25)
 shikimate kinase (EC 2.7.1.71) P10.2
 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EC 2.5.1.19)
 Chorismate synthase (EC 4.2.3.5)

Chorismate to tryptophan:

Anthranilate synthase (EC 4.1.3.27)
 Anthranilate phosphoribosyltransferase (EC 2.4.2.18)
 Phosphoribosyl anthranilate isomerase (EC 5.3.1.24)
 Indole-3-glycerol phosphate synthase (EC 4.1.1.48)
 Tryptophan synthase (EC 4.2.1.20)

Chorismate to phenylalanine/tyrosine

Chorismate mutase (EC 5.4.99.5)
 Prephenate decarboxylase (EC 4.2.1.51)
 Aromatic-amino-acid transaminase (EC 2.6.1.57)
 Tyrosine transaminase (EC 2.6.1.5)

Histidine (from PRPP and ATP):

ATP phosphoribosyltransferase (EC 2.4.2.17)
 Phosphoribosyl-ATP diphosphatase (EC 3.6.1.31)
 Phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19)
 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase (EC 5.3.1.16)
 Imidazole glycerol-phosphate synthase (EC 4.3.1.B2)
 Imidazoleglycerol-phosphate dehydratase (4.2.1.19)
 Histidinol-phosphate transaminase (EC 2.6.1.9)
 Histidinol-phosphatase (EC 3.1.3.15)
 Histidinol dehydrogenase (EC 1.1.1.23)

Amino acid degradation

Transaminase (EC 2.6.1.1, EC 2.6.1.2)
 Carbamoyl phosphate synthetase (EC 6.3.4.16)

Urea cycle

Ornithine transcarbamylase (EC 2.1.3.3)
 Argininosuccinate synthetase (EC 6.3.4.5)
 Argininosuccinate lyase (EC 4.3.2.1)
 Arginase (EC 3.5.3.1)

Alanine (*to pyruvate and glutamate*):

Alanine transaminase (EC 2.6.1.2)

Serine

to pyruvate:

Serine dehydratase (EC 4.3.1.17)

to glycine:

Serine hydroxymethyltransferase (EC 2.1.2.1)

Glycine (*glycine cleavage system*):

Glycine dehydrogenase (decarboxylating) (EC 1.4.4.2)

Aminomethyltransferase (EC 2.1.2.10)

Cysteine (*to pyruvate and SO₂*):

Cysteine dioxygenase (EC 1.13.11.20)

Aspartate transaminase (2.6.1.1)

Threonine

pathway 1 (to glycine and acetyl CoA):

Threonine dehydrogenase (EC 1.1.1.103)

Glycine C-acetyltransferase (EC 2.3.1.29)

pathway 2 (to glycine and acetaldehyde):

Threonine aldolase (EC 4.1.2.5)

pathway 3 (to succinyl-CoA via propionyl CoA):

Threonine dehydratase (EC 4.3.1.19)

2-oxobutanoate dehydrogenase (EC 1.2.4.4)

Propionyl-CoA carboxylase (EC 6.4.1.3)

Methylmalonyl-CoA mutase (EC 5.4.99.2)

Tryptophan (*to glutaryl-CoA*):

Tryptophan 2,3-dioxygenase (EC 1.13.11.11)

Acylformamidase (EC 3.5.1.19)

Kynurenine 3-monooxygenase (EC 1.14.13.9)

Kynureninase (EC 3.7.1.3)

3-hydroxyanthranilate 3,4-dioxygenase (EC 1.13.11.6)

Aminocarboxymuconate-semialdehyde decarboxylase (EC 4.1.1.45)

2-aminomuconate semialdehyde dehydrogenase (EC 1.2.1.32)

2-aminomuconate deaminase (EC 3.5.99.5)

2-oxoglutarate dehydrogenase (EC 1.2.4.2)

Asparagine (*to aspartate*):

Asparaginase (EC 3.5.1.1)

Aspartate*to oxaloacetate:*

Aspartate transaminase (EC 2.6.1.1)

to fumarate:

Aspartate-ammonia lyase (EC 4.3.1.1)

Glutamine (*to glutamate*):

Glutaminase (EC 3.5.1.2)

Glutamate (*to α -ketoglutarate*):

Glutamate dehydrogenase (EC 1.4.1.2)

Arginine (*to glutamate*):

Arginase (EC 3.5.3.1)

Ornithine transaminase (EC 2.6.1.13)

Glutamate semialdehyde dehydrogenase (EC 1.2.1.41)

Histidine (*to glutamate*):

Histidine ammonia-lyase (EC 4.3.1.3)

Urocanate hydratase (EC 4.2.1.49)

Imidazolonepropionase (EC 3.5.2.7)

Formimidoylglutamase (EC 3.5.3.8)

Valine, isoleucine, leucine:

Branched chain amino acid transaminase (EC 2.6.1.42)
 Branched chain ketoacid dehydrogenase complex (EC 1.2.4.4)
 Acyl CoA dehydrogenase (eg. EC 1.3.99.13)

Valine (to succinyl CoA):

Enoyl-CoA hydratase (EC 4.2.1.17)
 3-hydroxyisobutyryl-CoA hydrolase (EC 3.1.2.4)
 3-hydroxyisobutyrate dehydrogenase (EC 1.1.1.31)
 Methylmalonate-semialdehyde dehydrogenase (1.2.1.27)
 Propionyl-CoA carboxylase (EC 6.4.1.3)
 Methylmalonyl-CoA mutase (EC 5.4.99.2)

Isoleucine (to succinyl CoA and acetyl CoA)

Enoyl-CoA hydratase (EC 4.2.1.17)
 Methyl-hydroxybutyryl CoA dehydrogenase (EC 1.1.1.178)
 3-ketoacyl-CoA thiolase (EC 2.3.1.16)
 Propionyl-CoA carboxylase (EC 6.4.1.3)
 Methylmalonyl-CoA mutase (EC 5.4.99.2)

Leucine (to acetyl CoA)

Methylcrotonoyl-CoA carboxylase (EC 6.4.1.4)
 Methylglutaconyl-CoA hydratase (EC 4.2.1.18)
 Hydroxymethylglutaryl-CoA lyase (EC 4.1.3.4)

Methionine (to cysteine and succinyl-CoA):

Methionine adenosyltransferase (EC 2.5.1.6)
 Methyltransferase (eg. 2.1.1.37)
 Adenosylhomocysteinase (EC 3.3.1.1)
 Cystathionine beta-synthase (EC 4.2.1.22)
 Cystathionine gamma-lyase (EC 4.4.1.1)
 2-oxobutanoate dehydrogenase (EC 1.2.4.4)
 Propionyl-CoA carboxylase (EC 6.4.1.3)
 Methylmalonyl-CoA mutase (EC 5.4.99.2)

Lysine (to glutaryl-CoA):

Saccharopine dehydrogenase (EC 1.5.1.8)
 Saccharopine reductase (EC 1.5.1.10)
 Amino adipate-semialdehyde dehydrogenase (EC 1.2.1.31)
 2-amino adipate transaminase (EC 2.6.1.39)
 2-oxoglutarate dehydrogenase (EC 1.2.4.2)

Phenylalanine (*to tyrosine*):

Phenylalanine hydroxylase (EC 1.14.16.1)

Tyrosine (*to fumarate and acetoacetate*):

Tyrosine transaminase (EC 2.6.1.5)

4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27)

Homogentisate 1,2-dioxygenase (EC 1.13.11.5)

Maleylacetoacetate isomerase (EC 5.2.1.2)

Fumarylacetoacetase (EC 3.7.1.2)

Appendix II: Review of core mechanism types

As you work through the chapters in the second half of this book, you should be sure that you can recognize and draw general examples of all of the following core mechanism types.

Section 8.1: Nucleophilic substitution: S_N1 and S_N2

Section 9.2: Phosphate transfer

Section 10.2:

Formation of hemiacetal/hemiketal

Collapse of hemiacetal/hemiketal

Section 10.3:

Formation of acetal/ketal

Hydrolysis of acetal/ketal

Section 10.4: *N*-glycosidic bond formation and hydrolysis

Section 10.5: Imine formation and hydrolysis

Section 11.2 Nucleophilic acyl substitution at carboxylic acid derivatives

Section 11.8: Nucleophilic substitution at activated amides and carbamides

Section 12.2:

Carbonyl regioisomerization

Racemization/epimerization at α -carbon

Alkene regioisomerization

Section 12.3: Aldol addition / retroaldol cleavage

Section 13.1: Decarboxylation

Section 13.3: Claisen condensation / retro-Claisen cleavage

Section 13.4:

Conjugate (Michael) addition

$E1c_b$ β -elimination

Section 13.5: Carboxylation

Section 14.1: Electrophilic addition to alkene

Section 14.2: $E1$ β -elimination

Section 14.3: Electrophilic isomerization

Section 14.4: Electrophilic substitution

Section 14.5: Carbocation rearrangement

Section 15.3:

Hydrogenation aldehyde/ketone/imine by NAD(P)H

Dehydrogenation of alcohol/amine by NAD(P)⁺

Section 15.4:

Hydrogenation of conjugated alkene by NAD(P)H or FADH₂

Dehydrogenation of alkane by FAD

Section 15.6: Disulfide exchange

Section 16.2: Radical chain reactions